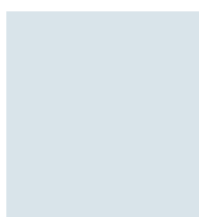


**The Centre for Veterinary Epidemiology and Risk Analysis**  
**The TB Diagnostics and Immunology Research Centre**  
**The Badger Vaccine Project**

*Biennial Report, 2010-11*



The Centre for Veterinary Epidemiology and Risk Analysis  
The TB Diagnostics and Immunology Research Centre  
The Badger Vaccine Project

## **Biennial Report, 2010-11**

*S.J. More and D.M. Collins (editors)*

*H.K. More (illustrations)*

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## Preface

The Department of Agriculture, Food and the Marine (DAFM) provides ongoing financial support to three research units within the UCD School of Veterinary Medicine at University College Dublin:

- The Centre for Veterinary Epidemiology and Risk Analysis (CVERA);
- The TB Diagnostics and Immunology Research Centre; and
- The Badger Vaccine Project.

These units each work to support DAFM policy, inspectorate and laboratory staff in the area of animal health. The TB Diagnostics and Immunology Research Centre and the Badger Vaccine Project focus on bovine tuberculosis research. CVERA is a national resource centre, providing policy advice and conducting epidemiological research on a wide range of animal health issues. In addition, CVERA provides general support to government, industry and the veterinary profession (pre- and post-graduation).

This report documents work conducted by, or in association with, these three UCD-based research units during 2010 and 2011.

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## Acknowledgements

### *The Centre for Veterinary Epidemiology and Risk Analysis*

CVERA works closely with colleagues from a wide range of organisations, both in Ireland and internationally, and their input is gratefully acknowledged. Staff from each of the following organisations were co-authors, with CVERA staff, of international peer-reviewed scientific papers published during 2010-11.

#### **University College Dublin**

- UCD College of Agriculture, Food Science and Veterinary Medicine
- UCD Institute for Food and Health
- UCD School of Applied Social Science
- UCD Geary Institute
- UCD School of Mathematical Sciences
- UCD School of Public Health, Physiotherapy and Population Science

#### **Rest of the Republic of Ireland**

- Animal Health Ireland
- Cork County Council
- Department of Agriculture, Food and the Marine (DAFM)
- DAFM Veterinary Laboratory Service
- Emlagh Lodge Veterinary Centre
- Enfer Scientific
- MSD Animal Health
- Private practitioners and consultants
- TCD School of Natural Sciences
- Teagasc
- The Irish Cattle Breeding Federation
- UCC School of Biological, Earth and Environmental Sciences

#### **United Kingdom**

- Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, Aberystwyth, Wales
- Institute of Evolutionary Biology, University of Edinburgh, Edinburgh, Scotland
- Institute of Life Sciences, School of Medicine, Swansea University, Swansea, Wales
- Peter Gorer Department of Immunobiology, Kings College London, London, England
- School of Biological Sciences, University of Edinburgh, Edinburgh, Scotland
- Scottish Agricultural College, Edinburgh and Midlothian, Scotland
- The Roslin Institute, Midlothian, Scotland
- Veterinary Epidemiology and Economics, Hertfordshire, England
- The Royal Veterinary College, England
- Veterinary Laboratories Agency, Weybridge, England

#### **Rest of World**

- Department of Large Animal Sciences, The Royal Veterinary and Agricultural University, Frederiksberg C, Denmark
- Department of Medicine, Mount Sinai School of Medicine, New York, U.S.A.
- Department of Population Medicine, University of Guelph, Ontario, Canada
- Food Safety and Sanitation Division, Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea
- National Veterinary Research and Quarantine Service, Republic of Korea
- Wageningen University, Wageningen, The Netherlands

## *The TB Diagnostics and Immunology Research Centre*

Staff from the centre acknowledge the help and support of District Veterinary Office (DVO) staff in providing samples for the IFN- $\gamma$  test.

## *The Badger Vaccine Project*

Staff working on the Badger Vaccine Project acknowledge the contribution and support of Kevin Kenny, Frances Quigley and colleagues at the mycobacteriology laboratory (DAFM Veterinary Laboratory Service, Backweston, Celbridge, Co. Kildare, Ireland), and Paddy Sleeman of University College Cork for fieldcraft. Glyn Hewinson, Mark Chambers, Sandrine Lesellier, and staff at the Animal Health Veterinary Laboratories Agency (AHVLA, UK) are also thanked for developing and carrying out many of the immunoassays used in the badger vaccine studies, and for contributing technical expertise and advice for the research programme.

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## Further information

In this report, projects are either:

- *Complete*, which includes those projects where relevant peer-reviewed papers, or equivalent, have been published in 2010/11, or
- *Current*, which includes the balance of active projects covering the spectrum from an advanced research concept through to final write-up.

Manuscript preparation is conducted in accordance with Uniform Requirements for Manuscripts Submitted to Biomedical Journals of the International Committee of Medical Journal Editors (previously the Vancouver Group). For further information, see [www.icmje.org](http://www.icmje.org). Guidelines for the transparent reporting of specific study types (for example, the CONSORT statement for transparent reporting of trials, [www.consort-statement.org](http://www.consort-statement.org)) are followed.

An up-to-date list of all peer-review papers produced by, or in association with, the Centre for Veterinary Epidemiology and Risk Analysis, the TB Diagnostics and Immunology Research Centre and the Badger Vaccine Project is available at [www.ucd.ie/cvera](http://www.ucd.ie/cvera).

## Affiliated staff members

### *The Centre for Veterinary Epidemiology and Risk Analysis*

#### **Board of management**

- Simon J. More (UCD)

#### *School, College and University representatives*

- University VP for Research
- Principal, UCD College of Agriculture, Food Science and Veterinary Medicine
- Head of UCD School of Veterinary Medicine
- Professor Michael Doherty, UCD School of Veterinary Medicine

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- State Veterinary Service:
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  - Michael Sheridan
  - Margaret Good
- Veterinary Laboratory Service
  - Dónal Sammin
- DAFM policy
  - Philip Carroll

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### *The Badger Vaccine Project*

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 Leigh Corner  
 Marion Barrett  
 Denise Murphy  
 Elvira Ramovic  
 Marian Teeling  
 Anthony Duignan (DAFM)  
 Eamon Costello (DAFM Veterinary Laboratory Service)

## Overview

### *The Centre for Veterinary Epidemiology and Risk Analysis*

The UCD Centre for Veterinary Epidemiology and Risk Analysis (UCD CVERA) is the national resource centre for veterinary epidemiology in Ireland, located within the UCD School of Veterinary Medicine at University College Dublin. The Centre was initially established as the Tuberculosis Investigation Unit, but in recent years has broadened its remit to cover a wide range of international, national and local animal health matters, including:

- Epidemiological support for the control and eradication of regulatory animal diseases, which includes national programmes for bovine tuberculosis, bovine brucellosis and bovine spongiform encephalopathy;
- Work in support of Animal Health Ireland ([www.animalhealthireland.ie](http://www.animalhealthireland.ie)), which is providing a proactive, coordinated and industry-led approach in Ireland to non-regulatory animal health concerns (such as mastitis, fertility and infectious bovine rhinotracheitis); and
- Epidemiological support for a broad range of other animal health and welfare issues relating to emergency animal disease preparedness and response (for example, avian influenza, bluetongue and equine infectious anaemia), on-farm investigations, welfare of farmed livestock and horses, health of companion animals and farmed fish, and international collaboration.

UCD CVERA staff work closely with national policy-makers, both in government and industry. Staff also contribute to training in veterinary medicine, both to undergraduates and postgraduate. A broad range of expertise is represented within the Centre, including agriculture and animal sciences, database development and management, geographic information systems, statistics, veterinary medicine and epidemiology. The Centre is staffed by employees of University College Dublin and of the Department of Agriculture, Food and the Marine (DAFM).

### *The Badger Vaccine Project*

The badger vaccine project is a programme of research with the objective to develop a vaccine to control tuberculosis in badgers and to break the link of infection to cattle. In studies with captive badgers, we have demonstrated that vaccination of badgers with BCG by a number of routes, including oral delivery, generates high levels of protective immunity against challenge with *M. bovis*. We are continuing to carry out studies with captive population of badgers to refine the vaccine and address issues relating to the eventual licensing of the vaccine as a veterinary medicine. We are also evaluating diagnostic tests with colleagues at AHVLA (Weybridge UK). A field trial commenced in 2009 to test the efficacy of the oral BCG vaccine in free-living badgers over a wide geographic area in Co. Kilkenny. The vaccination phase of the field trial will be completed in 2012, followed by detail analysis of the results. It is hoped that the data and experience generated during the field trial will lead to implementation of a vaccination strategy within the national control programme.



## *TB Diagnostics and Immunology Research Centre*

The gamma-interferon (IFN- $\gamma$ ) assay is used as a tool to assist in the eradication of bovine tuberculosis from the national cattle herd. All of the testing is carried out in the laboratory based at UCD. In the period 2010-2011, 13,662 blood samples were submitted to the laboratory for testing. The majority of samples originated from bovine reactor re-test herds, where the test was used to identify infected animals that were missed by the skin test. Other strategic uses of the test were targeted at inconclusive reactor re-tests and confirmation of the exposure status of skin test positive animals. The number of samples submitted was fewer than in 2008-2009 (28,000 samples); this was partly due to the reduced number of infected animals in the recent years. In addition, the focus of testing was targeted at the highest at-risk exposed cohorts of animals, based on epidemiological investigation. This resulted in identification of a higher proportion of IFN- $\gamma$  positive animals among the samples submitted for testing. In 2008-2009, 26% of samples submitted were positive to the IFN- $\gamma$  test. In 2010-2011 this increased to 33%, demonstrating a more efficient and cost-effective usage of the test. The laboratory continues to conduct research with a view to improving the performance of the assay under Irish conditions. Recent studies have investigated the performance, in Irish cattle, of an improved assay platform developed by Prionics Ltd. Results of this analysis are expected in 2012.









## Bovine tuberculosis

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## Improving surveillance

### Field-based surveillance

Using latent class analysis to estimate the test characteristics of the interferon- $\gamma$  test, the single intradermal comparative tuberculin test and a multiplex immunoassay under Irish conditions

Clegg, T.A.<sup>1</sup>, Duignan, A.<sup>2</sup>, Whelan, C.<sup>3</sup>, Gormley, E.<sup>4</sup>, Good, M.<sup>2</sup>, Clarke, J.<sup>3</sup>, Toft, N.<sup>5</sup>, More, S.J.<sup>1, 4</sup>

<sup>1</sup> UCD CVERA, <sup>2</sup> DAFM, <sup>3</sup> Enfer Scientific, <sup>4</sup> UCD School of Veterinary Medicine, <sup>5</sup> Department of Large Animal Sciences, The Royal Veterinary and Agricultural University, Frederiksberg C, Denmark

**Veterinary Microbiology 151, 68-76 (2011)**

Considerable effort has been devoted to improving the existing diagnostic tests for bovine tuberculosis (single intradermal comparative tuberculin test [SICTT] and  $\gamma$ -interferon assay [ $\gamma$ -IFN]) and to develop new tests. Previously, the diagnostic characteristics (sensitivity, specificity) have been estimated in populations with defined infection status. However, these approaches can be problematic as there may be few herds in Ireland where freedom from infection is guaranteed. We used latent class models to estimate the diagnostic characteristics of existing (SICTT and  $\gamma$ -IFN) and new (multiplex immunoassay [Enferplex-TB]) diagnostic tests under Irish field conditions where true disease status was unknown. The study population consisted of herds recruited in areas with no known TB problems (2,197 animals) and herds experiencing a confirmed TB breakdown (2,740 animals). A Bayesian model was developed, allowing for dependence between SICTT and  $\gamma$ -IFN, while assuming independence from the Enferplex-TB test. Different test interpretations were used for the analysis: SICTT (standard and severe interpretation),  $\gamma$ -IFN (a single interpretation), and a range of interpretations for the Enferplex-TB (level-1 [high sensitivity interpretation] to level-5 [high specificity interpretation]). The sensitivity and specificity (95% posterior credibility intervals; 95% PCI) of SICTT[standard] relative to Enferplex-TB[level-1] and  $\gamma$ -IFN were 52.9-60.8% and 99.2-99.8%, respectively. Equivalent estimates for  $\gamma$ -IFN relative to Enferplex-TB[level-1] and SICTT were 63.1-70.1% and 86.8-89.4%, respectively. Sensitivity of Enferplex-TB[level-1] (95% PCI: 64.8-71.9%) was superior to the SICTT[standard], and specificity of the Enferplex-TB[level-5] was superior to  $\gamma$ -IFN (95% PCI: 99.6-100.0%). These results provide robust measures of sensitivity and specificity under field conditions in Ireland and suggest that the Enferplex-TB test has the potential to improve on current diagnostics for TB infection in cattle. The extent of that potential will be assessed in further studies.

*Reprinted from Veterinary Microbiology, 151, Clegg, T.A., Duignan, A., Whelan, C., Gormley, E., Good, M., Clarke, J., Toft, N., More, S.J., Using latent class analysis to estimate the test characteristics of the interferon- $\gamma$  test, the single intradermal comparative tuberculin test and a multiplex immunoassay under Irish conditions, 68-76, Copyright 2011, with permission from Elsevier.*

### A follow up study of the $\gamma$ -interferon test results from the latent class analysis

Clegg, T.A.<sup>1</sup>, More, S.J.<sup>1, 2</sup>, Gormley, E.<sup>2</sup>

<sup>1</sup> UCD CVERA, <sup>2</sup> UCD School of Veterinary Medicine

The objective of this study is to identify risk factors associated with animals falsely testing positive to the gamma-interferon test. The study will follow animals from a very low bTB prevalence area that were tested using gamma-interferon in 2008. These animals were followed until the end of 2010 to identify any that were 'true' bTB positives in the follow-up period. Animals that were found to be bTB negative will be examined to identify risk factors, such as age or location, that may be associated with testing (falsely) positive to the gamma-interferon test in 2008.

## Primary isolation of *Mycobacterium bovis* from bovine tissues: Conditions for maximising the number of positive cultures

Corner, L.A.L.<sup>1</sup>, Gormley, E.<sup>1</sup>, Pfeiffer, D.U.<sup>2</sup>

<sup>1</sup> UCD School of Veterinary Medicine, <sup>2</sup> Royal Veterinary College, Hertfordshire, United Kingdom

### Veterinary Microbiology (in press)

In studies of *Mycobacterium bovis* infection in animals a definitive diagnosis requires the isolation of the organism. However, the optimum conditions for the primary isolation of *M. bovis* have not been determined. The aim of this study was to determine for primary isolation of *M. bovis*, (a) the incubation time required to achieve maximum sensitivity (i.e., the number of positive samples identified), (b) the effect of decontaminants on bacterial growth rates, and (c) the influence of media and the number of slopes of media on the number of positive samples detected. Two agar-based media, modified Middlebrook 7H11 (7H11) and tuberculosis blood agar (B83), and an egg-based medium, Stonebrink's (SB) were compared. Three decontaminants, 2% (w/v) sodium hydroxide (NaOH), 0.75% (w/v) and 0.075% (w/v) cetylpyridinium chloride (CPC, also called hexadecylpyridinium chloride, HPC) and 0.5% (w/v) benzalkonium chloride (BC) were evaluated against treatment with sterile distilled water. The inoculated media slopes were incubated for up to 15 weeks. Colonies first appeared after 2 weeks on all media types and 75% of positive slopes were identified by 8 weeks. An incubation time of 15 weeks was required to identify all positive samples. The slowest growth was associated with inocula that contained the fewest viable bacilli. The time to the appearance of colonies was influenced by medium type: the median time to detection of colonies was 28 days on 7H11 and B83, and 36 days on SB. However, SB returned the greatest number of positive samples. Decontamination procedures increased the minimum incubation time required to detect positive cultures, probably due to the toxic effect of the decontaminants. Increasing the number of inoculated slopes resulted in an increased number of positive samples and a decreased time to the detection of colonies. Overall, the detection of *M. bovis* was significantly influenced by the choice of media, the decontaminant and the duration of incubation of cultures.

*Reprinted from Veterinary Microbiology, Corner, L.A.L., Gormley, E., Pfeiffer, D.U., Primary isolation of Mycobacterium bovis for bovine tissues: Conditions for maximising the number of positive cultures, in press, Copyright 2012, with permission from Elsevier.*

## The comparative performance of the single intradermal test and the single intradermal comparative tuberculin test in Irish cattle, using tuberculin PPD combinations of differing potencies

Good, M.<sup>1</sup>, Clegg, T.A.<sup>2</sup>, Costello, E.<sup>3</sup>, More, S.J.<sup>2,4</sup>

<sup>1</sup> DAFM, <sup>2</sup> UCD CVERA, <sup>3</sup> DAFM Veterinary Laboratory Service, <sup>4</sup> UCD School of Veterinary Medicine

### The Veterinary Journal 190, e60-e65 (2011)

In national bovine tuberculosis (bTB) control programmes, testing is generally conducted using a single source of bovine purified protein derivative (PPD) tuberculin. Alternative tuberculin sources should be identified as part of a broad risk management strategy as problems of supply or quality cannot be discounted. This study was conducted to compare the impact of different potencies of a single bovine PPD tuberculin on the field performance of the single intradermal comparative tuberculin test (SICTT) and single intradermal test (SIT). Three trial potencies of bovine PPD tuberculin, as assayed in naturally infected bovines, namely, low (1192 IU/dose), normal (6,184 IU/dose) and high (12,554 IU/dose) were used. Three SICTTs (using) were conducted on 2,102 animals. Test results were compared based on reactor-status and changes in skin-thickness at the bovine tuberculin injection site. There was a significant difference in the

number of reactors detected using the high and low potency tuberculin. In the SICTT, high and low potency tuberculin detected 40% more and 50% fewer reactors, respectively, than normal potency tuberculin. Furthermore, use of the low potency tuberculin in the SICTT failed to detect 20% of 35 animals with visible lesions, and in the SIT 11% of the visible lesion animals would have been classified as negative. Tuberculin potency is critical to the performance of both the SICTT and SIT. Tuberculin of different potencies will affect reactor disclosure rates, confounding between-year or between-country comparisons. Independent checks of tuberculin potency are an important aspect of quality control in national bTB control programmes.

*Reprinted from The Veterinary Journal, 190, Good, M., Clegg, T.A., Costello, E., More, S.J., The comparative performance of the single intradermal test and the single intradermal comparative tuberculin test in Irish cattle, using tuberculin PPD combinations of differing potencies, e60-e65, Copyright 2011, with permission from Elsevier.*

## The comparative performance of the single intradermal comparative tuberculin test in Irish cattle, using tuberculin PPD combinations from different manufacturers

Good, M.<sup>1</sup>, Clegg, T.A.<sup>2</sup>, Murphy, F.<sup>1</sup>, More, S.J.<sup>2,3</sup>

<sup>1</sup> DAFM, <sup>2</sup> UCD CVERA, <sup>3</sup> UCD School of Veterinary Medicine

**Veterinary Microbiology 151, 77-84 (2011)**

Ireland currently obtains its avian and bovine tuberculin purified protein derivatives (PPDs) from a single source. Because problems of supply or quality cannot be discounted, it is prudent that Ireland identify alternative supplier(s) as part of a broad risk management strategy. Therefore, the aim of this study was to compare the performance of a number of different tuberculin combinations (that is, pairings of bovine and avian PPD; with different manufacturers) in the single intradermal comparative tuberculin test (SICTT), as currently performed in Ireland. The study was randomised, controlled and double-blinded. A total of 2,172 cattle were used in the study. Each animal was tested using two SICTTs, the first based on the tuberculin combination in current use, and the second using one of six trial tuberculin combinations. Analyses were conducted to compare both reactor-status and skin increase. For each control/trial tuberculin combination, there was good agreement between the control and trial reactor-status. Differences in skin increases were mainly confined to animals categorised as either negative or severe inconclusive. However, the measured differences were minor, and unlikely to have a significant impact on the actual test outcome, either for individual animals or for herds. In conclusion, while further studies determining sensitivity and specificity in Ireland would have to be done in the event of a change in tuberculin PPD there should be minimal disruption of the national programme if alternative tuberculin PPDs meeting WHO, OIE and EU regulations were used. In this study, the precision of the guinea pig bio-assay to assess tuberculin potency was low and therefore Ireland should maintain its practice of periodically assessing potency in naturally infected cattle, even though this is not currently required under WHO, OIE or EU Regulations.

*Reprinted from Veterinary Microbiology, 151, Good, M., Clegg, T.A., Murphy, F., More, S.J., The comparative performance of the single intradermal comparative tuberculin test in Irish cattle, using tuberculin PPD combinations from different manufacturers, 77-84, Copyright 2011, with permission from Elsevier.*



## Evaluation of the methodological quality of studies of the performance of diagnostic tests for bovine tuberculosis in cattle using QUADAS adapted for veterinary use

Downs, S.H.<sup>1</sup>, More, S.J.<sup>2,3</sup>, Broughan, J.M.<sup>1</sup>, Goodchild, A.V.<sup>1</sup>, Abernethy, D.A.<sup>4</sup>, Cameron, A.<sup>5</sup>, Cook, A.J.<sup>1</sup>, de la Rua-Domenech, R.<sup>6</sup>, Greiner, M.<sup>7</sup>, Gunn, J.<sup>8</sup>, Nuñez-Garcia, J.<sup>1</sup>, Rhodes, S.<sup>1</sup>, Rolfe, S.<sup>9</sup>, Sharp, M.<sup>10</sup>, Upton, P.<sup>1</sup>, Vordermeier, H.M.<sup>8</sup>, Watson, E.<sup>8</sup>, Welsh, M.<sup>11</sup>, Whelan, A.O.<sup>8</sup>, Woolliams, J.A.<sup>12</sup>, Parry, J.E.<sup>1</sup>, Clifton-Hadley, R.S.<sup>1</sup>

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A systematic review was conducted to identify studies that measured the performance of diagnostic tests for bTB in cattle. There has been little assessment of the methodological quality of these studies despite the importance of these tests in national surveillance and control schemes. Reference papers that appeared to have eligible data were reviewed using the QUADAS (Quality Assessment of Diagnostic Accuracy Studies) instrument adapted for veterinary use. During the study 190 references published between 1934 and 2009 were assessed using VETQUADAS by at least one of 18 reviewers. Of these, 107 were scored by 2 reviewers and 83 also had eligible estimates of sensitivity and or specificity for the systematic review. A similar pattern in the degree to which methodological criteria were met was observed across studies of different types of diagnostic tests. In references scored by two reviewers items measuring internal validity were assessed as having been met in 31-83% of studies. In only 31 and 45% of studies respectively did reviewers assess that the index test was interpreted without knowledge of the reference standard and the reference standard interpreted without knowledge of the index test. The review showed that there is considerable scope for improvement in the methodological quality of studies measuring performance of diagnostic tests for bTB.



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## Systematic review to identify primary research estimating the performance of diagnostic tests for bovine tuberculosis in cattle

Downs, S.H.<sup>1</sup>, Parry, J.E.<sup>1</sup>, Nuñez-García, J.<sup>1</sup>, Broughan, J.M.<sup>1</sup>, Abernethy, D.A.<sup>2</sup>, Cameron, A.R.<sup>3</sup>, Cook, A.J.<sup>1</sup>, de la Rua-Domenech, R.<sup>4</sup>, Goodchild, A.V.<sup>1</sup>, Greiner, M.<sup>5</sup>, Gunn, J.<sup>6</sup>, More, S.J.<sup>7,8</sup>, Pritchard, E.<sup>6</sup>, Rhodes, S.<sup>6</sup>, Rolfe, S.<sup>9</sup>, Sharp, M.<sup>10</sup>, Vordermeier, H.M.<sup>6</sup>, Watson, E.<sup>1</sup>, Welsh, M.<sup>11</sup>, Whelan, A.O.<sup>6</sup>, Woolliams, J.A.<sup>12</sup>, Upton, P.U.<sup>1</sup>, Clifton-Hadley, R.S.<sup>1</sup>

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The aim of this study was to conduct a systematic review to identify studies that have estimates of the sensitivity and specificity of diagnostic tests for bTB in cattle that could be used in a statistical meta-analysis of test performance. Detailed review was conducted by a working group of 18 reviewers. Comprehensive search criteria were developed and the process of review standardised. No limits were applied by year, language, region or type of diagnostic test in the initial search. 9,782 references were identified initially and abstracts, where available, were each reviewed by two reviewers. Entire references of those that passed through the initial review were randomly allocated to reviewers for detailed review if English language ( $n=215$ ) and to native speaker reviewers if non-English language ( $n=46$ ). An agreed range of data was extracted. There were 119 references with eligible performance estimates (published 1934-2009) for one or more of 14 different diagnostic tests. Studies varied in cattle population selection, reference standard and the thresholds and scales over which performance was measured. Methods for identifying studies that contain performance data for diagnostic tests for bTB are unspecific. Information provided in abstracts of studies of test performance needs to be standardised. Large scale studies of the performance of diagnostic tests for bTB in cattle are still needed.

## Results from a meta-analysis of sensitivity and specificity of diagnostic tests for bovine tuberculosis in cattle

Nuñez-García, J.<sup>1</sup>, Downs, S.H.<sup>1</sup>, Parry, J.E.<sup>1</sup>, Abernethy, D.A.<sup>2</sup>, Broughan, J.M.<sup>1</sup>, Cameron, A.R.<sup>3</sup>, Cook, A.J.<sup>1</sup>, de la Rua-Domenech, R.<sup>4</sup>, Goodchild, A.V.<sup>1</sup>, Greiner, M.<sup>5</sup>, Gunn, J.<sup>6</sup>, More, S.J.<sup>7,8</sup>, Pritchard, E.<sup>6</sup>, Rhodes, S.<sup>6</sup>, Rolfe, S.<sup>9</sup>, Sharp, M.<sup>10</sup>, Upton, P.U.<sup>1</sup>, Vordermeier, H.M.<sup>6</sup>, Watson, E.<sup>1</sup>, Welsh, M.<sup>11</sup>, Whelan, A.O.<sup>6</sup>, Woolliams, J.A.<sup>12</sup>, Clifton-Hadley, R.S.<sup>1</sup>

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A meta-analysis study was conducted using data extracted during a systematic review of references that reported the sensitivity (Se) and/or specificity (Sp) of diagnostic tests for bovine tuberculosis (bTB) in cattle. Performances were estimated, for 14 different diagnostic tests and modifications of the tests, using binary logistic regression models adjusting for relevant covariates. Parameters in the models were estimated using Monte Carlo Markov Chain with the category best representing test conditions in Great Britain and in Ireland as baseline. Estimates for the most common



tests used in GB for the interferon gamma blood test using bovine and bovine-avian PPD were 0.87 (95% Bayesian Credible Interval 0.72, 0.95) and 0.67 (95% BCI 0.49, 0.82), respectively, for Se and 0.97 (95% BCI 0.94, 0.98) and 0.98 (95% BCI 0.96, 0.99) for Sp and for the single intradermal comparative cervical tuberculin skin test using standard interpretation were 0.50 for Se (95% BCI 0.26, 0.78) and 1 (95% BCI 0.99, 1.00) for Sp.

## Bovine tuberculosis: Effect of the tuberculin skin test on *in vitro* interferon gamma responses

Schiller, I.<sup>1</sup>, Vordermeier, H.M.<sup>2</sup>, Waters, W.R.<sup>3</sup>, Whelan, A.O.<sup>2</sup>, Coad, M.<sup>2</sup>, Gormley, E.<sup>4</sup>, Buddle, B.M.<sup>5</sup>, Palmer, M.<sup>3</sup>, Thacker, T.<sup>3</sup>, McNair, J.<sup>6</sup>, Welsh, M.<sup>6</sup>, Hewinson, R.G.<sup>2</sup>, Oesch, B.<sup>7</sup>

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### Veterinary Immunology and Immunopathology 136, 1–11 (2010)

Bovine tuberculosis (bTB) is a disease of zoonotic and economic importance. In many countries, control is based on test and slaughter policies and/or abattoir surveillance. For testing, cell mediated immune- (CMI-) based assays (i.e., tuberculin skin test (TST) supplemented by the interferon gamma (IFN-gamma) assay) are the primary surveillance and disease control tests for bTB. The combined use of the *in vivo* and *in vitro* CMI assays to increase overall sensitivity has raised the question of whether the IFN-gamma response is influenced by injection of purified protein derivatives (PPDs) for TST. Published data on the influence of the TST, applied as the caudal fold test (CFT) or the comparative cervical test (CCT), on the IFN-gamma assay are contradictory. Reviewing published data and including additional data, the following conclusions can be drawn: (1) in naturally infected cattle, PPD administration for the single or repeated short-interval CCT neither boosts nor depresses PPD-specific IFN-gamma production. Disparate results have been concluded from some studies using experimental infections, emphasizing the importance of confirming initial experimental-based findings with studies using cattle naturally infected with *Mycobacterium bovis*. (2) In cattle experimentally infected with *M. bovis*, PPD administration for CFT boosts PPD-specific IFN-gamma production for up to 7 days without any effect on test interpretation. Importantly, in naturally infected cattle, CFT-related boosting selectively increases the *in vitro* *M. bovis* PPD (PPD-B) response 3 days after CFT, resulting in an increased PPD-B response relative to the response to *Mycobacterium avium* PPD (PPD-A). In non-infected cattle, it cannot be excluded that the CFT induces a mild boost of the PPD-specific response, particularly in animals sensitized to environmental, non-tuberculous mycobacteria, thus decreasing the specificity of the IFN-gamma assay. (3) In general, there is a lack of data clearly characterizing the effect of TSTs on the IFN-gamma assay. Further studies are required to clearly describe the effects of both CFT and CCT in non-infected animals and in naturally infected cattle, especially in low reacting infected cattle.

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## Development and evaluation of an enzyme-linked immunosorbent assay for use in the detection of bovine tuberculosis in cattle

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### Clinical and Vaccine Immunology 18, 1882-1888 (2011)

As a consequence of continued spillover of *Mycobacterium bovis* into cattle from wildlife reservoirs and increased globalization of cattle trade with associated transmission risks, new approaches such as vaccination and novel testing algorithms are seriously being considered by regulatory agencies for the control of bovine tuberculosis. Serologic tests offer opportunities for identification of *M. bovis* infected animals not afforded by current diagnostic techniques. The present study describes assay development and field assessment of a new commercial ELISA that detects antibody to *M. bovis* antigens MPB83 and MPB70 in infected cattle. Pertinent findings include: specific antibody responses were detected ~90-100 days after experimental *M. bovis* challenge, minimal cross-reactive responses were elicited by infection/sensitization with non-tuberculous *Mycobacteria* spp., and the apparent sensitivity/specificity of the ELISA with naturally infected cattle was 63% / 98% respectively, with sensitivity improving as disease severity increased. The ELISA also detected infected animals missed by the routine tuberculin skin test and antibody was detectable in bulk tank milk samples from *M. bovis*-infected dairy herds. A high throughput ELISA could be adapted as a movement, border, or slaughter surveillance test, as well as a supplemental test to tuberculin skin testing.

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## Specificity of the tuberculin test in cattle herds adjacent to peat-land areas in Ireland

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In Ireland, where peat-lands occur on the margins of grassland, there is some concern that cattle may become sensitized to environmental mycobacteria, and result in false positive reaction to the tuberculin skin test, or a so called non-specific reactor (NSI). This is evidenced by the finding of low lesion rates in reactors from certain areas. To date, little work has been done to assess this issue of test specificity. This study describes the relationship between visible lesion rates at slaughterhouse among standard reactor animals and likelihood of exposure to environmental mycobacteria at pasture adjacent to peat land, within Co. Tipperary, over the period 2007-2009. The aim of this study is to model the likelihood of detecting a post mortem lesion at slaughter in standard reactors derived from herds grazing within 500m of peat land (exposed) compared to herds grazed more than 2km away from peat-land (unexposed). Landuse will be determined by reference to the Corine™ dataset, using ArcGIS software. We will analyze the data at the animal level. Data will be analysed using a multivariable logistic regression model with the presence of a visible lesion at slaughter as the positive outcome. Animals showing no visible lesion (NVL) will have negative outcomes. The exposure of interest will be proximity to peat-land. Other explanatory variables will include previous animal and herd-level bTB test readings, and other nationally available data.

## Factory surveillance

### Relative effectiveness of Irish factories in the surveillance of slaughtered cattle for visible lesions of tuberculosis, 2005-2007

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#### Irish Veterinary Journal (in press)

In Ireland, as part of the ongoing bovine tuberculosis (bTB) control/eradication scheme, every animal is examined at slaughter for its fitness for human consumption. The aim of this study was to determine the relative effectiveness of factories in submitting and subsequently in having these suspect lesions confirmed as bTB lesions during the years 2005-2007 in Ireland and to provide an update from previously published data for years 2003-2004. During 2005-2007 data were available on 4,401,813 cattle from attested herds, from which data for potential confounding factors was available for 3,344,057 animals slaughtered at export-licensed factories, of which there were 37 during that period. From these animals, 8,178 suspect lesions were submitted for laboratory confirmation. Lesions from 5,456 (66.7%) animals were tested as positive, 2,453 (30%) as negative and 269 (3.2%) were inconclusive for bTB. Logistic regression was used to determine adjusted submission and confirmation risks for each factory while controlling for confounding factors. Factory rankings based on adjusted and crude risks were similar. The average crude submission risk for all the factories was 25 lesions per 10,000 animals slaughtered, ranging from 0 to 52. The crude confirmation risk varied between 30.3% and 91.3%. Substantial variation between surveillance effectiveness in the 37 factories was found. Compared to previous years (2003-2004), there was an increased bTB lesion submission and confirmation risk. Continued monitoring of the effectiveness of slaughter surveillance in Ireland is recommended; emphasis should be placed on efforts to improve bTB surveillance in factories with lower rankings.

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### Evaluation of a risk-based approach to meat inspection for bovine tuberculosis in Ireland

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The aim of this study was to consider the risk factors for the finding of suspect bovine tuberculosis (bTB) lesions at post mortem meat inspection in Ireland. Tuberculin testing results for bTB and laboratory submission of suspect post mortem bTB lesions, were analysed. The year 2009 was selected for analysis in which 1,515,217 bovines were slaughtered in Ireland. Multivariable logistic regression analysis of the data showed that the age of the animal was a good predictor of the presence of bTB lesions at meat inspection. In animals under the age of three, the suspect lesion identification rate is less than half the rate for animals aged three and above. The impact of a risk-based meat inspection system for bTB in Ireland, i.e. by a reduced meat inspection system for younger bovines, was considered.

## Improving management of high risk herds

### *Introduced infection*

#### Study of herds that introduced new animals during the course of a restriction for bovine tuberculosis

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During a movement restriction for bovine tuberculosis, a farmer may wish to buy new cattle in order to either replace animals lost as a result of bovine tuberculosis (bTB) or to enable routine management practices to continue 'as normal' (in spite of the bTB restrictions imposed). Permission to allow the restocking of restricted herds is currently an area of policy uncertainty, due to the unknown risks of the purchased animals. The objectives of this study are i) to determine whether restocking during restriction is associated with increased bTB risk, ii) to provide an overview of events associated with each restocked episode and to clarify the infection status of animals that are introduced during restrictions and iii) on the proviso that there is evidence of an increased bTB risk, to identify the practices relating to restocking that are the most risky and to determine whether the increased risk is associated with the source or the restocked herd.

#### Predictors of the first between-herd animal movement for cattle born in 2002 in Ireland

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#### **Preventive Veterinary Medicine 97, 264-269 (2010)**

Movement of animals between farms represents a potential risk of bovine tuberculosis (bTB) and other disease transmission. The objectives of this study were to identify and quantify risk factors associated with the first between-herd movement of animals (denoted as risk move). A random sample of 1 percent of Irish calf births registered for 2002 (20,182 animals) was selected. Descriptive and survival analysis on movement over the period 2002-2005 was performed. A total of 12,119 (60%) of animals experienced a risk move over the 4-year study period. Among those that moved, 57% did so within the first 12 months of age. For animals in dairy herds, an early peak in risk move events was observed within the first 12 weeks of age; whereas in animals from suckler herds, a later risk move peak was observed between 21 and 36 weeks of age. The survival models identified a number of risk factors: two that appeared most important in predicting a risk move were gender and enterprise type. Males had a hazard ratio of 2.6 times that of females. The hazards for enterprise type, varied over time, thus a time-varying covariate ( $\text{ent\_type} \times \ln(\text{time})$ ) was included in the Cox model. At 7 days of age, females in suckler herds were at 0.14 times the hazard of females in dairy herds for risk move, and over time, the hazards converged, equalised by day 140, and then diverged, so that by 4 years of age, females in suckler herds were at 4.64 times the hazard of females in dairy herds. Herds with a history of selling animals in previous years maintained that record during the study period with increased hazard of risk move. Enterprise type interacted with gender so that relative to females, males from dairy herds were at greater hazard of risk move than males from suckler herds. Hazard of risk move was also a function of  $\ln(\text{herd area})$ , so that each doubling of farm area was accompanied by a 30.6% decrease in the hazards. The main conclusion was that risk of movement related disease transmission also depends on the purpose of the movement whether for breeding or for beef finishing. While males were at greater

hazard of movement than females, they would have a shorter lifespan, thus limiting the opportunity for further transmission post-movement.

*Reprinted from Preventive Veterinary Medicine, 97, White, P.W., Frankena, K., O’Keeffe, J., More, S.J., Martin, S.W., Predictors of the first between-herd animal movement for cattle born in 2002 in Ireland, 264-269, Copyright 2010, with permission from Elsevier.*

## Local TB persistence

### Risk of bovine tuberculosis for cattle sold out from attested herds during year 2005 in Ireland: a descriptive analysis

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Bovine tuberculosis (bTB) is an infectious contagious disease caused by a bacterium, *Mycobacterium bovis* (*M. bovis*). The significance of bTB lies in trade implications and zoonotic potential. Although industrialized countries have significantly reduced the prevalence of *M. bovis* infection in both humans and cattle, bTB persists in some developed countries, including the Republic of Ireland. A retrospective cohort study was conducted to determine the risk of bTB among animals sold out from “clear” herds during the year 2005. Herds from which the animals were sold out were classified as “exposed” and “non-exposed” (our main risk factor) to bTB according to their previous bTB history during the year 2005. Our study sample was comprised of 338,960 animals, from which 124,360 were sold out from “exposed” herds and 214,600 animals were sold out from “non-exposed” herds. The overall risk of bTB during the 2-year period after the animals were sold out was 0.69%. The odds of testing positive to bTB was 1.94 (95% CI = 1.79-2.11,  $p = <0.0001$ ) for animals sold out from “exposed” herds compared to animals sold out from “non-exposed” herds. Other confounding factors (age and gender) and bTB breakdown severity during 2005 were included in our analysis and had a significant association with the risk of bTB at the animal level in Ireland. This type of analysis should in the near future incorporate other risk factors in order to demonstrate quality assurance that can be supported, tracked and guaranteed.

### A retrospective cohort study of the risk of TB among suckler calves whose dam tested positive to the single intradermal comparative tuberculin test (SICTT)

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The very close relationship of a suckling dam with her offspring and their contact via the suckling process and grooming raises the question of whether or not an infected dam could possibly transmit tuberculosis to her offspring via either the oral or respiratory route. The aim of this study is to assess the hypothesis that calves whose dams failed the Single Intradermal Comparative Tuberculin Test (SICTT) will have an increased risk of failing the test in the future compared to those whose dams were negative to the SICTT. A retrospective cohort study was designed. Data from all suckler herds which failed the SICTT test in Co. Clare in 2007 will be obtained from Animal Health Computer System (AHCS) operated by the Department of Agriculture, Food and the Marine (DAFM). A control sample of suckler cows negatives to the skin test and their respective calves will be also obtained.

## Shorter-term risk of *Mycobacterium bovis* in Irish cattle following an inconclusive diagnosis to the single intradermal comparative tuberculin test

Clegg, T.A.<sup>1</sup>, Good, M.<sup>2</sup>, Duignan, A.<sup>2</sup>, Doyle, R.<sup>2</sup>, More., S.J.<sup>1, 3</sup>

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### Preventive Veterinary Medicine 102, 255-264 (2011)

In Ireland, new cases of bovine tuberculosis (bTB) are detected using both field and abattoir surveillance (More and Good, 2006). Field surveillance is conducted through annual testing of all cattle using the single intradermal comparative tuberculin test (SICTT). An animal may be deemed a 'standard inconclusive reactor' (SIR) to the SICTT if the bovine response is >2 mm and between 1 and 4 mm > the avian response. The herdowner then has three choices for the management of the SIR: option 1 is to have the animal retested after a minimum period of 42 days (an inconclusive reactor retest, IRR), option 2 is to slaughter the SIR and, provided the animal has no visible lesions, have a full herd test 42 days after the SIR leaves the herd, option 3 is to slaughter the SIR and have the lymph nodes examined using histology and/or culture for bTB. In the current study, we examine the bTB risk for SIRs both at slaughter prior to the IRR and at the IRR, and the future bTB risk of TIR animals (so-called 'transient SIRs'; SIR animals with a negative SICTT result at the subsequent IRR) that moved from the herd of disclosure within 6 months of the IRR. We also investigate factors associated with the future bTB status of SIRs at slaughter prior to the IRR and at the IRR. The study population included all SIRs identified in Ireland between 2005 and 2009 inclusive in a herd otherwise Officially TB free (OTF). Between 11.8% and 21.4% of SIRs slaughtered prior to the IRR were confirmed bTB positive at post mortem (using histology or culture if histology was not definitive), compared to 0.13–0.22% of SICTT –ve cohort animals. The post mortem bTB lesion rate of SIRs is lower than the lesion rate reported for reactor animals between 2005 and 2009 of between 34% and 39%, reflecting the doubtful infection status of these animals. Between 20.3% and 27.9% of herds were restricted at the IRR. The herd restriction rate amongst the national herd between 2005 and 2009 varied from 5.09% to 6.02%. TIRs that moved out of the disclosing herd within 6 months of the IRR were 12 times more likely to be bTB positive at the next test/slaughter compared to all animals in the national herd. The same increased risk did not apply to the SICTT –ve cohort animals that moved out of the same herds at the same time. Based on a range of measures, SIRs and TIRs are each at increased bTB risk into the future. Consequently, differential treatment of TIR animals would be justified.

*Reprinted from Preventive Veterinary Medicine, 102, Clegg, T.A., Good, M., Duignan, A., Doyle, R., More., S.J., Shorter-term risk of Mycobacterium bovis in Irish cattle following an inconclusive diagnosis to the single intradermal comparative tuberculin test, 255-264, Copyright 2011, with permission from Elsevier.*

## Longer-term risk of *Mycobacterium bovis* in Irish cattle following an inconclusive diagnosis to the single intradermal comparative tuberculin test

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### Preventive Veterinary Medicine 100, 147-154 (2011)

In Ireland, new bovine tuberculosis (bTB) cases are detected using both field and abattoir surveillance. During field surveillance, an animal may be deemed a 'standard inconclusive reactor' (SIR) to the single intradermal comparative tuberculin test (SICTT) if the bovine response is >2mm, and from 1 to 4mm greater than the avian response. Little is known about the future infection risk posed by SIR animals that pass a subsequent retest, so-called 'transient SIR' (TIR) animals. The objective of this study was to critically evaluate the future bTB status of TIR animals, by examining the



future risk of bTB diagnosis over the 4 years following initial SIR diagnosis and clearance at the subsequent retest. The study included all TIRs that were identified as SIRs in 2005 in otherwise free herds at tests with no other reactors at that test and that were clear at the subsequent retest. The analysis was restricted to cows that were neither sold, other than direct to slaughter, nor exported from the herd during the follow up period (to the end of 2009). Five control cows were randomly selected from each study herd. A parametric survival model with shared frailties, to account for clustering within herds, was developed to model time from passing a retest to future bTB diagnosis. The final parametric survival model contained the variables: TIR status in 2005, inconclusive status during the follow-up period, location, herd restricted during the study, time since last restriction within the herd and age. The time ratio for the TIR status variable was significant ( $p < 0.001$ ) indicating that on average the time to diagnosis with bTB for TIRs was 78% shorter compared to the non-TIRs. The frailty term was significant ( $p < 0.001$ ) indicating that animals within some herds were more likely to become reactors compared to other herds. These results have important implications for national policy and future management of TIR animals. Further, private veterinary practitioners and their clients should be aware of the increased risk associated with TIRs.

*Reprinted from Preventive Veterinary Medicine, 100, Clegg, T.A., Good, M., Duignan, A., Doyle, R., Blake, M., More, S.J., Longer-term risk of Mycobacterium bovis in Irish cattle following an inconclusive diagnosis to the single intradermal comparative tuberculin test, 147-154, Copyright 2011, with permission from Elsevier.*

## Effect of distance between a previously *Mycobacterium bovis*-infected herd and clear herds on the occurrence of bovine tuberculosis breakdowns in Ireland

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This study looks at herds surrounding a recently derestricted herd and seeks to answer the following question - what is the animal level bTB risk in the 4 calendar years following derestriction? We will conduct a retrospective cohort study, comparing herds that were (case herds) and were not (control herds) derestricted in 2005 (case herds). The study will focus on future animal test outcomes, up to end of 2008, for animals present within neighbouring herds. Herd-level explanatory variables (for case herds) include number of reactors, number of standard reactors, indices of length of restriction, and herd size at the 2005 completed episode. Among herds surrounding case herds, a matching number of control herds will be selected at random within county, as one from each of the following zones: i: From contiguous herds within 25m (direct neighbours) of a derestricted herd; ii: From contiguous herds within 26m-150m of a derestricted herd; iii: From contiguous herds within 151m – 1km of a derestricted herd. Each comparison herd will be randomly assigned an end date of restriction within 2005, stratified by month, to follow the same distribution as the case herds. For each case herd, and control herds, a list of animals present at the 2005 derestriction date will be reconstituted from CMMS records (the “study population”). Subsequent animal test outcomes for the study population will be compared up to the end of 2008 between animals from case vs. control herds.



## Risk of bTB associated with animals moving out of derestricted herds

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This study seeks to answer the following question - for herds that were recently derestricted, what is the animal level bTB risk for animals sold to other herds compared to those remaining within the herds? We will conduct a retrospective cohort study. For herds derestricted in 2005 (case herds), a comparison of future animal test outcomes will be made with herds that remained unrestricted (control herds). Herd-level explanatory variables for case herds include number of reactors, number of standard reactors, indices of length of restriction, and herd size at the 2005 completed episode. For each case herd, a matching number of control herds will be selected from herds that remained unrestricted in 2005 and preceding years as follows: Group1: unrestricted during 2004 and 2005, Group2: unrestricted during 2003, 2004 and 2005 and Group3: unrestricted during 2002, 2003, 2004 and 2005. Each comparison herds will be randomly assigned an end date of restriction within 2005, allowing for seasonal variations to follow the same distribution as the case herds. For each case herd, and control herd, a list of animals present at the 2005 derestriction date will be reconstituted from CMMS records (the "study population"). Details of subsequent between-herd movements will be extracted from CMMS for the period up to end of 2008. Those moving out of their study herd to another herd will be classed as "movers", and the remainder classed as "non-movers". By means of a survival analysis, animal test outcomes for the study population will be compared over time between movers vs. non-movers reconstituted from case and control herds.

## The temporal and spatial patterns of bovine tuberculosis in County Kilkenny cattle herds, 1998 to 2008

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The use of epidemiological methods is an intrinsic component of disease management and control. The objective of this study is to examine Bovine Tuberculosis (bTB) in County Kilkenny cattle herds from 1998 to 2008 inclusive and use the information to enhance the understanding of the epidemiology of the disease in the county. The study will examine the long term changes that have occurred over the decade (secular trends), and will investigate cyclical disease patterns and seasonal disease patterns. Spatial disease patterns may be visualised and analysed by using cartographic (mapping) or geographic information systems (GIS) methods.

## Impact of the national full herd depopulation policy on the recurrence of bovine tuberculosis in Irish herds, 2003 to 2005

Good, M.<sup>1</sup>, Clegg, T.A.<sup>2</sup>, Duignan, A.<sup>1</sup>, More, S.J.<sup>2,3</sup>

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### Veterinary Record 169, 581 (2011)

This study evaluated the impact of the Irish herd bovine tuberculosis (bTB) depopulation policy (depopulation, disinfection, contiguous testing and local badger removal where implicated) on the recurrence of bTB infection, by comparing the future risk in restocked herds following depopulation for either bTB or bovine spongiform encephalopathy (BSE) during 2003 to 2005. Each herd was assigned a 'previous bTB risk', based on bTB history during the five years before depopulation. Future bTB risk was estimated, using a multivariable Cox proportional hazard model for time-to-breakdown for each study herd, to identify risk factors associated with bTB. Future bTB risk varied significantly by reason for depopulation and previous bTB risk. Herds depopulated for bTB (by definition, at high bTB risk) were not significantly different from BSE herds with no or a low previous bTB risk. BSE herds with a high previous bTB risk were found to be at significantly greater future bTB risk. Herd bTB depopulation measures, as currently applied in Ireland, are shown to be effective in enabling herds to attain and retain bTB freedom following restocking. Based on the data presented, and consistent with current knowledge of the bTB epidemiology, local badger removal contributes to efforts to limit recurrence of bTB in Ireland.

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## The association between weather and bovine tuberculosis

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In a time when it is widely acknowledged that climate change is occurring globally, the issue of weather in relation to the epidemiology of bovine TB needs greater understanding. For the period 2005-2009, we examine the influence of weather variables on TB incidence in cattle herds together with well established risk factors in the area of west County Wicklow in the east of Ireland which until recently had a separate district veterinary office (DVO). Data for this study were obtained from three sources: Data from the national database of TB testing history (VetNet) maintained by the Department of Agriculture, Food and the Marine (DAFM), meteorological data from the Irish Meteorological Office and badger data from the Wildlife Unit of DAFM all for west County Wicklow. The data will be analysed using statistical tools related to generalized linear mixed models and time series. Initial results suggest that high rainfall is associated with increased bTB levels in cattle herds.

## Estimation of the between-herd reproduction ratio for contiguous spread of bovine tuberculosis

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Bovine tuberculosis (bTB) is a recurring disease burden for the Irish cattle sector, with between 20,000-30,000 reactors being removed annually under the national herd testing program. While GIS techniques have demonstrated that clustering of bTB exists in cattle herds on a wider spatial scale, relatively little work has been published on the risk of bTB transmission among herds within 25m (i.e. directly contiguous) of one other. Neighbouring herds within 25m of a herd experiencing a new bTB episode (index herd) may come into contact with bTB infected cattle and could become infected with bTB as a result. Alternatively, both index and neighbouring herds could be exposed to a common infected wildlife source. The pattern of contiguous spread among herds within areas of differing badger density may provide some useful insights into the nature bTB clustering. The study population will consist of herds within Removal (lower badger density) and Reference (higher badger density) areas of the Four Area Project, comprising parts of Cork, Donegal, Kilkenny and Monaghan. By use of herd testing data for the period of proactive badger removal, between August 1997 and September 2002, we will identify herds experiencing one or more bTB episodes (index herds). Using LPIS data for 2001, we will determine contiguous herds within 25m of an index herd, and assess the previous bTB history of those (contiguous) herds as a potential source of bTB exposure for the index herd. Using this information we will develop a mathematical model to calculate the between-herd reproduction ratio ( $R_0$ ) for a bTB episode among neighbouring herds at 25m. The key factor of interest as a determinant of  $R_0$  will be treatment area (removal or reference).

## How important is neighbourhood spread in the persistence of bovine tuberculosis in Irish cattle herds?

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A key feature of bovine tuberculosis (bTB) is local persistence of infection, characterised by recurrent episodes in one or more neighbouring herds. Local persistence could be attributed to: residual infection that persists in the index herd, despite testing following initial disclosure, neighbourhood or contiguous spread by cattle-to cattle transmission over farm boundaries, and/or infection of herds via a common wildlife source, e.g. the badger. A case-control study was conducted on the association between the occurrence of a bTB episode in cattle herds in the Republic of Ireland, and a number of possible risk factors; the bTB history of neighbouring herds within 1km, the herds own bTB history, herd size, enterprise type, number of farm fragments, and the purchasing of cattle. Neighbouring herds were divided into three zones based on their closest association with a study (own) herd as: zone 1 (0-25m), zone 2 (26-150m) and zone 3 (151-1000m). The characteristics of 3,909 herds that had a bTB episode in 2006 were compared with those of 98,872 herds that were bTB tested, and remained clear throughout 2006. The results highlight an association between bTB and an increased animal incidence within two subsets of neighbouring herds: (i) herds directly contiguous during the previous 2 years, and (ii) herds at a distance of > 25 metres in the previous year. Further studies will be necessary to determine to what extent the association at (i) may be confounded by the existence of a wildlife (badger) source.

## Predictive models

### Stochastic herd-level modelling of freedom from bTB infection for testing strategies applied in differing risk scenarios in Great Britain

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The performance of surveillance system components (including diagnostic tests and slaughterhouse inspection), which make up a bovine tuberculosis surveillance system were evaluated using a stochastic model. This first calculates the probability that a herd, with specific characteristics and bTB surveillance history, is free from infection. The sensitivity of further surveillance required to meet a 'target' probability of freedom from infection is then calculated and subsequent decisions on the selection of components, which offer adequate sensitivity, can be based on specificity (expected number of false positives), time to achieve freedom, and therefore cost. The model required several inputs including evidence from previous herd surveillance, the prior probability that the herd was infected (estimated using local prevalence of bTB), the distributions of animal-level sensitivity and specificity of diagnostic tests (derived from a meta-analysis of diagnostic test performance) and the risk of introduction of infection into the herd (derived from the observed incidence of bTB in GB risk scenarios). Where the probability of introduction of infection was high, it was difficult to achieve and maintain a high target probability of herd freedom from infection even with the most sensitive of components. Conversely, where introduction of infection was low, components with low sensitivity may still provide adequate probability of freedom from TB.

## From explanation to prediction: developing a predictive model for recurrent bovine tuberculosis in Irish cattle herds

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### Preventive Veterinary Medicine 94, 170-177 (2010)

There is a good understanding of factors associated with bovine tuberculosis (bTB) risk in Irish herds. As yet, however, this knowledge has not been incorporated into predictive models with the potential for improved, risk-based surveillance. The goal of the study was to enhance the national herd scoring system for bTB risk, thus leading to improved identification of cattle herds at high risk of recurrent bTB episodes. A retrospective cohort study was conducted to develop a statistical model predictive of recurrent bovine tuberculosis episodes in cattle herds in the Republic of Ireland. Herd-level disease history data for the previous 12 years, the previous 3 years, the previous episode, and the current-episode were used in survival analyses to determine the aspects of disease history that were predictive of a recurrent breakdown within 3 years of a cleared bTB episode. Relative to herds with 0-1 standard reactors in the current bTB episode, hazard ratios increased to 1.3 and 1.6 for herds with 2-5 and >5 standard reactors, respectively. Compared to herds with <30 animals, hazard ratios increased from 1.8 to 2.5 and then to 3.1 for herds with 30-79, 80-173, and >174 animals respectively. Relative to herds with <4 herd-level tests in the previous 3 years, herds with 4-5 and >5 tests had 1.1 and 1.4 times greater hazard of a bTB breakdown. Herds that did not have a bTB episode in the 5 years prior to their 2001 episode were 0.8 times less likely to breakdown in the next 3 years than herds that did. Herds breaking down in the spring or summer were 0.8 times less likely to suffer a recurrent breakdown than herds breaking down in autumn or winter (this was likely due to seasonality in testing regimes). The presence of a confirmed bTB lesion was not predictive of increased risk of recurrent bTB. Despite the availability of detailed disease history, the predictive ability of the model was poor. One explanation for this was that herds suffering a recurrence of bTB on their first test after clearing a bTB episode were different from herds that broke down later in the period at risk. Future research might need to include additional variables to identify which subsets of herd bTB episodes, if any, have identifiable features that are predictive of recurrent breakdowns.

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## Supporting studies

### Genetics, milk production

#### Evidence for genetic variance in resistance to tuberculosis in Great Britain and Irish Holstein-Friesian populations

Bermingham, M.L.<sup>1</sup>, Brotherstone, S.<sup>2</sup>, Berry, D.P.<sup>3</sup>, More, S.J.<sup>4,5</sup>, Good, M.<sup>6</sup>, Cromie, A.R.<sup>7</sup>, White, I.M.S.<sup>2</sup>, Higgins, I.M.<sup>4</sup>, Coffey, M.<sup>8</sup>, Downs, S.H.<sup>9</sup>, Glass, E.J.<sup>1</sup>, Bishop, S.C.<sup>1</sup>, Mitchell, A.P.<sup>9</sup>, Clifton-Hadley, R.S.<sup>9</sup>, Woolliams, J.A.<sup>1</sup>

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#### BMC Proceedings 5 (Suppl 4), S15 (2011)

Here, we jointly summarise scientific evidence for genetic variation in resistance to infection with *Mycobacterium bovis*, the primary agent of bovine tuberculosis (TB), provided by two recent and separate studies of Holstein-Friesian dairy cow populations in Great Britain (GB) and Ireland. The studies quantified genetic variation within archived data from field and abattoir surveillance control programmes within each country. These data included results from the single intradermal comparative tuberculin test (SICTT), abattoir inspection for TB lesions and laboratory confirmation of disease status. Threshold animal models were used to estimate variance components for responsiveness to the SICTT and abattoir confirmed *M. bovis* infection. The link functions between the observed 0/1 scale and the liability scale were the complementary log-log in the GB, and logit link function in the Irish population. The estimated heritability of susceptibility to TB, as judged by responsiveness to the SICTT, was 0.16 (0.012) and 0.14 (0.025) in the GB and Irish populations, respectively. For abattoir or laboratory confirmation of infection, estimates were 0.18 (0.044) and 0.18 (0.041) from the GB and the Irish populations, respectively. Estimates were all significantly different from zero and indicate that exploitable variation exists among GB and Irish Holstein Friesian dairy cows for resistance to TB. Epidemiological analysis suggests that factors such as variation in exposure or imperfect sensitivity and specificity would have resulted in underestimation of the true values.

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#### Genetic correlations between measures of *Mycobacterium bovis* infection and economically important traits in Irish Holstein-Friesian dairy cows

Bermingham, M.L.<sup>1</sup>, More, S.J.<sup>2,3</sup>, Good M.<sup>4</sup>, Cromie, A.R.<sup>5</sup>, Higgins, I.M.<sup>2</sup>, Berry, D.P.<sup>1</sup>

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#### Journal of Dairy Science 93, 5413-5422 (2010)

*Mycobacterium bovis* is the primary agent of tuberculosis (TB) in cattle. The failure of Ireland and some other countries to reach TB-free status indicates a need to investigate complementary control strategies. One such approach would be genetic selection for increased resistance to TB. Previous research has shown that considerable genetic variation exists for susceptibility to the measures of *M. bovis* infection, confirmed *M. bovis* infection, and *M. bovis*-purified protein derivative (PPD) responsiveness. The objective of this study was to estimate the genetic and phenotypic correlations

between economically important traits and these measures of *M. bovis* infection. A total of 20,148 and 17,178 cows with confirmed *M. bovis* infection and *M. bovis*-PPD responsiveness records, respectively, were available for inclusion in the analysis. First - to third-parity milk, fat, and protein yields, somatic cell count, calving interval, and survival, as well as first-parity body condition score records, were available on cows that calved between 1985 and 2007. Bivariate linear-linear and threshold-linear sire mixed models were used to estimate (co)variance components. The genetic correlations between economically important traits and the measures of *M. bovis* infection estimated from the linear-linear and threshold-linear sire models were similar. The genetic correlations between susceptibility to confirmed *M. bovis* infection and economically important traits investigated in this study were all close to zero. *Mycobacterium bovis*-PPD responsiveness was positively genetically correlated with fat production (0.39) and body condition score (0.36), and negatively correlated with somatic cell score (-0.34) and survival (-0.62). Hence, selection for increased survival may indirectly reduce susceptibility to *M. bovis* infection, whereas selection for reduced somatic cell count and increased fat production and body condition score may increase susceptibility to *M. bovis* infection.

*Reprinted from Journal of Dairy Science 93, Bermingham, M.L., More, S.J., Good M., Cromie, A.R., Higgins, I.M., Berry, D.P., Genetic correlations between measures of Mycobacterium bovis infection and economically important traits in Irish Holstein-Friesian dairy cows, 5413-5422, Copyright (2010), with permission from the American Dairy Science Association.*

## Genotype by environment interaction of susceptibility to *Mycobacterium bovis* infection and economically important traits in Irish Holstein-Friesian cows across areas of low and high TB incidence

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*Mycobacterium bovis* is the primary agent of tuberculosis in cattle. There is considerable genetic variation for susceptibility to *M. bovis* infection, and both favorable and antagonistic genetic correlations exist between susceptibility to *M. bovis* infection and health, fertility and production traits in Irish Holstein-Friesian dairy cattle. The effectiveness of breeding program for increased resistance to TB and performance in other economically important traits may be hindered via genotype by environment interaction, whereby different genotypes respond differently to changes in the environment. The objective of this study was therefore to identify the existence and scope of genotype by environment interaction for susceptibility to *M. bovis* infection and other economically important traits of Holstein-Friesian dairy cattle across low and high TB incidence areas in Ireland. The single intradermal comparative tuberculin test (SICTT) was used as a measure of susceptibility of cows to *M. bovis* infection. A total of 19,892 cows with tuberculin test records between 2000 and 2008 were available for inclusion in the analysis. First to third parity milk, fat, and protein yield, somatic cell count, calving interval and survival, as well as first parity body condition score records, were available on cows calving between 1985 and 2007. Tuberculosis incidence in Ireland for the years 2000 to 2008 was mapped using Geographical Information Systems methodology. The data was split into two environments based on a SICTT reactor density threshold of 0.34 cattle per km<sup>2</sup> per year. Bivariate linear sire mixed models were used to estimate (co)variance components between traits across the high and low TB incidence environments. Homogeneous genetic variance was observed for variance response to the SICTT across the low and high incidence environments ( $P > 0.05$ ). The genetic correlation between response to the SICTT in the low and high incidence environment was not significantly less than unity ( $P > 0.05$ ); indicating that re-ranking of sires is limited. However, heterogeneous genetic correlations were estimated between three parity milk fat production and response to the SICTT across the high (0.30 - 0.35) and low (-0.10 - -0.02) incidence environments. Hence, selection for increased milk fat production will increase susceptibility to TB in high incidence areas. The absence of environmental sensitivity of response to the SICTT across environments indicates national level selection is feasible. The heterogeneous genetic correlation between milk fat production responses to the SICTT, however, needs to be considered in the design of breeding programs in order to accurately predict rate of genetic gain across TB incidence environments.



## Genetics of animal health and disease in cattle

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**Irish Veterinary Journal 64, 5 (2011)**

There have been considerable recent advancements in animal breeding and genetics relevant to disease control in cattle, which can now be utilised as part of an overall programme for improved cattle health. This review summarises the contribution of genetic makeup to differences in resistance to many diseases affecting cattle. Significant genetic variation in susceptibility to disease does exist among cattle suggesting that genetic selection for improved resistance to disease will be fruitful. Deficiencies in accurately recorded data on individual animal susceptibility to disease are, however, currently hindering the inclusion of health and disease resistance traits in national breeding goals. Developments in 'omics' technologies, such as genomic selection, may help overcome some of the limitations of traditional breeding programmes and will be especially beneficial in breeding for lowly heritable disease traits that only manifest themselves following exposure to pathogens or environmental stressors in adulthood. However, access to large databases of phenotypes on health and disease will still be necessary. This review clearly shows that genetics make a significant contribution to the overall health and resistance to disease in cattle. Therefore, breeding programmes for improved animal health and disease resistance should be seen as an integral part of any overall national disease control strategy.

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## Bovine tuberculosis and milk production in infected dairy herds in Ireland

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**Preventive Veterinary Medicine 93, 153-161 (2010)**

This study describes the relationship between bovine tuberculosis (TB) and milk yield in TB-infected dairy herds in Ireland. The study had two objectives: to determine whether cows detected as TB reactors (and thus subject to immediate slaughter) were likely to be the higher milk-producing cows, and to determine whether subclinical TB infection was associated with reduced milk production at or around the time of disclosure (detection). All Irish dairy herds restricted from trading between the 1<sup>st</sup> June 2004 and the 31<sup>st</sup> May 2005 as a result of two or more TB reactors by the Single Intradermal Comparative Tuberculin Test (SICTT) were considered for study. The data consisted of 419 herds. Data were collected on all TB reactors and a random sample of 5 non-reactor cows in these herds: a data set of 4,340 cows (2,342 TB reactors and 1,998 non-reactors). Previous milk data for the cows were taken into consideration and thus all lactations on a cow were analysed together with the years of lactations. There was an inherent hierarchical structure in the data, with lactations nested within cows and cows within herds and thus a linear mixed model with two random effects was used to describe the data. The results of this study showed that for all lactations and years under investigation, milk yield was significantly lower for TB reactor cows, with differences ranging from 120kg (2003, lactation 3) to 573kg (2001, lactation 1), when compared to the non-reactor cows.

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## Bovine tuberculosis and udder health in Irish dairy herds

Boland, F.<sup>1</sup>, Kelly, G.E.<sup>1</sup>, Good, M.<sup>2</sup>, More, S.J.<sup>3, 4</sup>

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### The Veterinary Journal (in press)

The association between bovine tuberculosis (TB) infection status based on results from the Single Intradermal Comparative Tuberculin Test (SICTT) and milk production has been described in dairy cows in TB-infected herds in Ireland. The biological basis was uncertain, but could be related to increased TB susceptibility among lower producing dairy cows. In this study, the relationship between somatic cell count (as an objective measure of udder health) and SICTT reactivity (as a proxy for TB infection status) was investigated. Somatic cell counts of TB infected cows, both during and prior to the lactation of diagnosis of TB infection, were examined and compared to non-infected cows. All Irish dairy herds restricted from trading between June 2004 and May 2005 as a result of two or more TB reactors (test positive) to the SICTT were considered for study. Data were collected on 4,340 cows from 419 herds. Previous lactation data for the cows were taken into consideration and all lactations of a cow were analysed together with the years of lactations. There was an inherent hierarchical structure in the data, with lactations nested within cows and cows within herds and so a linear mixed model with two random effects was used to describe the data. Milk production (305-day milk yield) was also included in the model as a fixed effect. The results of the study showed that for all lactations and years under investigation, somatic cell counts for SICTT reactor cows when compared to the non-reactor cows were not significantly different. In this study population, TB infection status was not associated with udder health.

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## Evidence of genetic resistance of cattle to infection with *Mycobacterium bovis*

Brotherstone, S.<sup>1</sup>, White, I.M.S.<sup>1</sup>, Coffey, M.<sup>2</sup>, Downs, S.H.<sup>3</sup>, Mitchell, A.P.<sup>3</sup>, Clifton-Hadley, R.S.<sup>3</sup>, More, S.J.<sup>4, 5</sup>, Good, M.<sup>6</sup>, Woolliams, J.A.<sup>7</sup>

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### Journal of Dairy Science 93, 1234-1242 (2010)

Anecdotal evidence points to genetic variation in resistance of cattle to infection with *Mycobacterium bovis*, the causative agent of bovine tuberculosis (bTB), and published experimental evidence in deer and cattle suggests significant genetic variation in resistance and reactivity to diagnostic tests. However, such genetic variation has not been properly quantified in the United Kingdom dairy cattle population; it is possible that it exists and may be a factor influencing the occurrence of bTB. Using models based on the outcome of the process of diagnosis (ultimate fate models) and on the outcome of a single stage of diagnosis (continuation ratio models, herd test-date models), this study shows that there is heritable variation in individual cow susceptibility to bTB, and that selection for milk yield is unlikely to have contributed to the current epidemic. Results demonstrate that genetics could play an important role in controlling bTB by reducing both the incidence and the severity of herd breakdowns.

*Reprinted from Journal of Dairy Science, 93, Brotherstone, S., White, I.M.S., Coffey, M., Downs, S.H., Mitchell, A.P., Clifton-Hadley, R.S., More, S.J., Good, M., Woolliams, J.A., Evidence of genetic resistance of cattle to infection with Mycobacterium bovis, 1234-1242, Copyright 2010, with permission from the American Dairy Science Association.*

## Genome-wide transcriptional profiling of peripheral blood leukocytes from cattle infected with *Mycobacterium bovis* reveals suppression of host immune genes

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**BMC Genomics 12, 611 (2011)**

*Mycobacterium bovis* is the causative agent of bovine tuberculosis (bTB), a pathological infection with significant economic impact. Recent studies have highlighted the role of functional genomics to better understand the molecular mechanisms governing the host immune response to *M. bovis* infection. Furthermore, these studies may enable the identification of novel transcriptional markers of bTB that can augment current diagnostic tests and surveillance programmes. In the present study, we have analysed the transcriptome of peripheral blood leukocytes (PBL) from eight *M. bovis*-infected and eight control non-infected age-matched and sex-matched Holstein-Friesian cattle using the Affymetrix® GeneChip® Bovine Genome Array with 24,072 gene probe sets representing more than 23,000 gene transcripts. Control and infected animals had similar mean white blood cell counts. However, the mean number of lymphocytes was significantly increased in the infected group relative to the control group ( $P = 0.001$ ), while the mean number of monocytes was significantly decreased in the bTB group ( $P = 0.002$ ). Hierarchical clustering analysis using gene expression data from all 5,388 detectable mRNA transcripts unambiguously partitioned the animals according to their disease status. In total, 2,960 gene transcripts were differentially expressed (DE) between the infected and control animal groups (adjusted  $P$ -value threshold  $\leq 0.05$ ); with the number of gene transcripts showing decreased relative expression (1,563) exceeding those displaying increased relative expression (1,397). Systems analysis using the Ingenuity® Systems Pathway Analysis (IPA) Knowledge Base revealed an over-representation of DE genes involved in the immune response functional category. More specifically, 64.5% of genes in the affects immune response subcategory displayed decreased relative expression levels in the infected animals compared to the control group. This study demonstrates that genome-wide transcriptional profiling of PBL can distinguish active *M. bovis*-infected animals from control non-infected animals. Furthermore, the results obtained support previous investigations demonstrating that mycobacterial infection is associated with host transcriptional suppression. These data support the use of transcriptomic technologies to enable the identification of robust, reliable transcriptional markers of active *M. bovis* infection.

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## Impact of delayed processing of bovine peripheral blood on differential gene expression

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**Veterinary Immunology and Immunopathology (in press)**

RT-qPCR can be used to accurately determine expression levels of genes following RNA extraction from tissue samples. If blood is the source of total RNA, it is often desirable to process the samples immediately following collection because delays in processing for RNA extraction may influence mRNA expression estimates obtained from RT-qPCR analyses.

However, this may not be feasible if the site of blood collection is distant from the processing laboratory. In the present study, the effects of delays in the processing of blood samples on mRNA expression data was investigated using a panel of 23 functionally diverse genes from five different gene ontology (GO) categories in peripheral blood sampled from ten age-matched healthy cattle. Venous blood was collected in Tempus™ Blood RNA tubes, which contain reagents that lyse blood cells immediately and stabilise the RNA signature (*T<sub>0</sub>*). Blood was also collected in conventional lithium heparin collection tubes, and stored at ambient temperature for *T<sub>4</sub>*, *T<sub>6</sub>* and *T<sub>8</sub>* h, prior to total RNA extraction. The mRNA expression profiles of these 23 genes were determined by RT-qPCR and compared across the time course. Thirteen genes showed significant up- or down-fold changes in mRNA expression over the 8 h time course. Among the GO categories, genes in the Immune Response category showed the most differential expression. These results also demonstrated that the changes in mRNA expression for the *IFNG* gene, which encodes the cytokine IFN- $\gamma$ , did not correspond to IFN- $\gamma$  protein levels estimated using ELISA.

*Reprinted from Veterinary Immunology and Immunopathology 145, Sheridan, M.P., Browne, J.A., MacHugh, D.E., Costello, E., Gormley, E., Impact of delayed processing of bovine peripheral blood on differential gene expression, 199-205, Copyright 2012, with permission from Elsevier.*

## Transcriptional profiling of immune genes in bovine monocyte-derived macrophages exposed to bacterial antigens

Taraktoglou, M.<sup>1</sup>, Szalabska, U.<sup>1</sup>, Magee, D.A.<sup>1</sup>, Browne, J.A.<sup>1</sup>, Sweeney, T.<sup>2</sup>, Gormley, E.<sup>3</sup>, MacHugh, D.E.<sup>1,4</sup>

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### Veterinary Immunology and Immunopathology 140, 130-139 (2011)

The involvement of Toll-like receptors (TLRs) and other immune signalling genes during challenge of bovine macrophages with bacterial products derived from disease-causing bacteria in cattle was investigated. An *in vitro* cell culture model of bovine monocyte derived macrophages (MDM) was established and these cells were exposed to purified protein derivative (PPD-b) derived from *Mycobacterium bovis* and to lipopolysaccharide (LPS) derived from *Escherichia coli*. Following 24h incubation, total RNA was extracted and expression of immune related genes was determined by real time quantitative reverse transcription PCR (qRT-PCR). Expression of a selection of genes spanning the TLR-2 and TLR-4 pathways, from the initial activation of the receptors to the production of pro-inflammatory cytokines and chemokines was determined. Results from repeat experiments using MDM from seven different age-matched dairy cattle showed that PPD-b treatment caused significant up-regulation of the *TLR2* and *TLR4* genes and the expression profile of TLR adaptor molecules suggested that this signalling is MYD88-dependent. Conversely, LPS caused significant up-regulation of *TLR4* via a MYD88-independent signalling pathway. Significant up-regulation of genes involved with NF- $\kappa$ B signalling was also detected in PPD-b- and LPS-treated samples accompanied by the expression of pro-inflammatory cytokine (*TNF*, *IL1B*, *IL6*) and chemokine genes (*IL8*, *CCL5*, *CCL3*). Overall, LPS challenge resulted in a more marked up-regulation of immune-related genes. Furthermore, the magnitude fold-change difference in gene expression suggests, at least in part, that bovine macrophages produce IFN- $\gamma$  as a result of LPS challenge.

*Reprinted from Veterinary Immunology and Immunopathology, 140, Taraktoglou, M., Szalabska, U., Magee, D.A., Browne, J.A., Sweeney, T., Gormley, E., MacHugh, D.E., Transcriptional profiling of immune genes in bovine monocyte-derived macrophages exposed to bacterial antigens, 130-139, Copyright 2011, with permission from Elsevier.*

## Tuberculosis in other farmed livestock species

### Granuloma encapsulation is a key factor for containing tuberculosis infection in minipigs

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**PLoS One 5, e10030 (2010)**

A transthoracic infection involving a low dose of *Mycobacterium tuberculosis* has been used to establish a new model of infection in minipigs. The 20-week monitoring period showed a marked Th1 response and poor humoral response for the whole infection. A detailed histopathological analysis was performed after slicing the formalin-fixed whole lungs of each animal. All lesions were recorded and classified according to their microscopic aspect, their relationship with the intralobular connective network and their degree of maturity in order to obtain a dissemination ratio (DR) between recent and old lesions. CFU counts and evolution of the DR with time showed that the proposed model correlated with a contained infection, decreasing from week 9 onwards. These findings suggest that the infection induces an initial Th1 response, which is followed by local fibrosis and encapsulation of the granulomas, thereby decreasing the onset of new lesions. Two therapeutic strategies were applied in order to understand how they could influence the model. Thus, chemotherapy with isoniazid alone helped to decrease the total number of lesions, despite the increase in DR after week 9, with similar kinetics to those of the control group, whereas addition of a therapeutic *M. tuberculosis* fragment-based vaccine after chemotherapy increased the Th1 and humoral responses, as well as the number of lesions, but decreased the DR. By providing a local pulmonary structure similar to that in humans, the mini-pig model highlights new aspects that could be key to a better understanding tuberculosis infection control in humans.

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## Tuberculosis in goats on a farm in Ireland: epidemiological investigation and control

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### Veterinary Record 168, 485 (2011)

This paper describes an outbreak of tuberculosis (TB) caused by *Mycobacterium bovis* in a dairy goat herd on a farm in Ireland, where 66.3 per cent of the herd tested positive to the single intradermal comparative tuberculin test (SICTT) at initial detection. An epidemiological investigation was conducted to determine the origin of the outbreak, considering issues such as animal movements and herd management practices. Infection was introduced with a consignment of goats, as determined by the variable number tandem repeat profile. Infection was eradicated using a test and cull programme involving the SICTT, the interferon- $\gamma$  assay and a multiplex immunoassay (Enferplex TB).

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## Application of the Enfer Chemiluminescent Multiplex ELISA System for the detection of *Mycobacterium bovis* infection in goats

Shuralev, E.<sup>1</sup>, Quinn, P.<sup>1</sup>, Doyle, M.<sup>2</sup>, Duignan, A.<sup>3</sup>, Kwok, H.F.<sup>4</sup>, Bezoz, J.<sup>5</sup>, Olwill, S.A.<sup>4</sup>, Gormley, E.<sup>2</sup>, Aranaz, A.<sup>5</sup>, Good, M.<sup>3</sup>, Davis, W.C.<sup>6</sup>, Clarke, J.<sup>1</sup>, Whelan, C.<sup>1</sup>

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### Veterinary Microbiology (in press)

A study was conducted to optimise a multiplex serological immunoassay for use in identification of goats infected with *Mycobacterium bovis*. To assess assay specificity, 31 goats with a history of being free from *M. bovis* infection were used. To determine assay sensitivity, 180 Single Intradermal Comparative Tuberculin test (SICTT) positive goats were recruited. Additionally, 286 SICTT negative goats classed as potentially exposed animals present in the same positive herds were also included in the study. The results of the assay demonstrated a specificity of 100%. The multiplex assay detected 57/60 SICTT (95.0%) positive animals in one *M. bovis* infected herd and 120/120 (100%) in a second herd. In a separate experiment, 28 *M. caprae* culture confirmed infected goats from Spain were assayed, of which 24 (85.7%) were found positive in the test. The results show that inclusion of an antibody based assay can improve the ability to identify *M. bovis* and *M. caprae* infected goats. With further development and validation the multiplex assay may prove to be a useful tool for control of *M. bovis* and *M. caprae* infection in goats.

*Reprinted from Veterinary Microbiology 154, Shuralev, E., Quinn, P., Doyle, M., Duignan, A., Kwok, H.F., Bezoz, J., Olwill, S.A., Gormley, E., Aranaz, A., Good, M., Davis, W.C., Clarke, J., Whelan, C., Application of the Enfer Chemiluminescent Multiplex ELISA System for the detection of Mycobacterium bovis infection in goats, 292-297, Copyright 2012, with permission from Elsevier.*

## Improved understanding of ecology and TB epidemiology

### Badger ecology

#### Can Eurasian badger (*Meles meles*) numbers be predicted from sett attributes and capture history? An application and evaluation of multivariable modelling

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Predicting badger numbers from sett characteristics and capture histories is of considerable applied importance. This ability would be useful in generating probable badger densities for disease and strategic models of bovine tuberculosis (bTB) control. Modelling is of interest to elucidate the factors that may impact on badger capture at local scales. Furthermore, badger management and vaccination programs would benefit by increasing the probability of efficiently capturing the target badger populations. Within this context, it will be investigated whether badger capture numbers can be predicted from field signs and previous capture histories. The relative benefits of different modelling approaches will also be explored (GLM, GEE, Zero-inflated with Poisson or Negative Binomial distributions). The different modelling techniques will be compared in terms of mean predicted error and coverage.

#### Impact of culling on relative abundance of the Eurasian badger (*Meles meles*) in three counties in Ireland

Byrne, A.W.<sup>1,2</sup>, O'Keeffe, J.<sup>3,5</sup>, Sleeman, D.P.<sup>2</sup>, Davenport, J.<sup>2</sup>, Martin, S.W.<sup>4</sup>

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The Eurasian badger (*Meles meles*) has been implicated in the epidemiology of bovine tuberculosis (bTB) in cattle populations in the Republic of Ireland. Badger populations have been subject to a regulated culling regime in areas with chronic histories of bTB cattle herd breakdowns. Removal data collected during this regime from 2004 to 2010 will be used to model the impact of culling on populations in areas under capture. Additionally, changes in field signs of badger activity will be used as an index of abundance to verify the outcomes of the removal models. The removal intensities will also be investigated and compared with previous experimental culls. These models will elucidate trends in badger population density over time in response to the culling regime.

#### The ecology of the Eurasian badger (*Meles meles*) in Ireland: a review

Byrne, A.W.<sup>1,2</sup>, Sleeman, D.P.<sup>2</sup>, O'Keeffe, J.<sup>3,4</sup>, Davenport, J.<sup>2</sup>

<sup>1</sup> National Biodiversity Data Centre, Waterford Institute of Technology, <sup>2</sup> UCC School of Biological, Earth and Environmental Sciences, <sup>3</sup> UCD CVERA, <sup>4</sup> DAFM

There has been extensive research on the ecology of the Eurasian badger in Ireland. Despite much of the recent literature focusing on disease (bovine tuberculosis) dynamics relating to badgers, a great deal of insight into the autecology of the species in Ireland has emerged. A study will be undertaken to review all relevant and available studies relating to Irish badger populations. Particular effort will be made to investigate 'grey literature' (non-peer

reviewed material, including national and departmental research reports and theses) - often ignored work that may contain valuable observations. The study will also compare the differences and similarities of badger populations both within, and outside of Ireland.

## Diet of the European badger (*Meles meles*) in the Republic of Ireland: A comparison of results from an analysis of stomach contents and rectal faeces

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### Mammalian Biology 76, 470-475 (2011)

The diets of mammals have been investigated primarily through the analysis of faecal samples. In our study we analysed both stomach contents, and rectal faeces from European badgers. This approach enabled a direct comparison of the information derived from these two sources. The dietary components found from each source were the same. However, it was found that, compared to stomach contents, the contribution to the diet, by volume, of plant litter, earthworms, Tipulid larvae and adult Carabid beetles were significantly overestimated by faecal analysis, while those of Noctuid larvae and Carabid beetle larvae were significantly underestimated. The analysis of stomach contents showed clear evidence of seasonality in the consumption of earthworms, Carabid beetle larvae, Tipulid larvae and Noctuid larvae. This seasonality was not as evident when the diet was inferred by the analysis of faeces. We propose that an analysis of stomach contents rather than of faeces, more accurately reflects the relative proportions of ingested food types, and the seasonality of the diet.

*Reprinted from Mammalian Biology, 76, Cleary, G.P., Corner, L.A.L., O’Keeffe, J., Marples, N.M., Diet of the European badger (Meles meles) in the Republic of Ireland: A comparison of results from an analysis of stomach contents and rectal faeces, 470-475, Copyright Deutsche Gesellschaft für Säugetierkunde 2011, with permission from Elsevier.*

## Analysis of movement patterns of satellite-tracked badgers in Co. Wicklow

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We have used home range data from satellite-tracked badgers to plot ‘Minimum Convex Polygon’ (MCP) home ranges for each badger in each month and have looked at the trends in average MCP values throughout the year. The results have shown that home ranges for the majority of individuals were greatest in summer and smallest in winter. This provides support for our current knowledge of the activity of badgers, as we know that they are relatively inactive in winter and much more active in summer. However, some individuals appear to use almost their whole summer range throughout the winter season, suggesting that there is greater variation in winter behaviour than has been found previously. We are also investigating whether age or sex have any noticeable effects on home range size. To do this we are using kernel estimates (generally considered to be the best assessment of home ranges) as well as MCPs, in order to get a more accurate picture of their behaviour. The satellite-tracking information on badger movements is also being used to study the effects of temperature and precipitation on the home ranges by comparing the data generated during a mild winter (2011-12) and a relatively cold winter (2010-2011). We are modelling the variation of MCP and kernel estimates of the badgers with age, sex, county, rainfall and temperature data. From these models we hope to identify the factors that best explain the variance in the dataset. Another study is looking at the frequency of badger incur-

sions into or near to farm buildings, and whether they actively avoid such incursions. We are also examining the preferences of badgers for foraging in different vegetation types. The results of this work will provide fundamental information on the nature of badgers' interactions with farms and may have implications for the transmission rates of tuberculosis from badgers to cattle.

## The effect of badger removal on road casualty mammals

O'Shea, F.<sup>1</sup>, Sleeman, D.P.<sup>2</sup>, Davenport, J.<sup>2</sup>

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**Irish Naturalists' Journal 31, 118-122 (2010)**

Records of a five year survey of mammalian road casualties in two different parts (removal and reference areas) of the Co. Cork portion of the Four Area Badger Project are reported. There were more hedgehog, domestic cat and fox casualties in the removal area, but only the first two species differed to a statistically significant extent from the reference area. How such studies might be improved and the possible implications for epidemiology and biodiversity are discussed.

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## Badger road casualties in rural areas of the Republic of Ireland

Sleeman, D.P.<sup>1</sup>, Collins, D.M.<sup>2</sup>, Davenport, J.<sup>1</sup>

<sup>1</sup> UCC School of Biological, Earth and Environmental Sciences, <sup>2</sup> UCD CVERA

Road casualties are a cause of mortality for badgers. This project will look at such casualties in relation to a number of previously estimated populations in rural areas in the Republic of Ireland.

## The badgers (*Meles meles* (L.)) of Little Island, Co. Waterford

Sleeman, D.P.<sup>1</sup>, Partridge, T.<sup>2</sup>, O'Boyle, I.<sup>2</sup>, Gormley, E.<sup>3</sup>, Toolan, D.<sup>4</sup>

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**Irish Naturalists' Journal 31, 94-99 (2010)**

A high density island population of badgers is described. The methods used in the study to capture, anaesthetize and identify badgers, as well as to map territories, are detailed. The island had eight main setts and six territories. One territory had three main setts, two of which had small sub-territories. Badgers captured in the initial study, and those found dead from 1998-2007, are detailed. The finding of badgers without tuberculosis which were unwell (emaciated, with heavy ectoparasite infestations), in buildings is recorded. The implications for badger research and disease management are discussed.

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## TB epidemiology

### Estimating the extent of spatial association of *Mycobacterium bovis* infection in badgers in Ireland

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**Epidemiology and Infection 138, 270-279 (2010)**

*Mycobacterium bovis* infects the wildlife species badgers *Meles meles* who are linked with the spread of the associated disease tuberculosis (TB) in cattle. Control of livestock infections depends in part on the spatial and social structure of the wildlife host. Here we describe spatial association of *M. bovis* infection in a badger population using data from the first year of the Four Area Project in Ireland. Using second-order intensity functions, we show there is strong evidence of clustering of TB cases in each the four areas, i.e. a global tendency for infected cases to occur near other infected cases. Using estimated intensity functions, we identify locations where particular strains of TB cluster. Generalized linear geostatistical models are used to assess the practical range at which spatial correlation occurs and is found to exceed 6 in all areas. The study is of relevance concerning the scale of localized badger culling in the control of the disease in cattle.

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### Spatial clustering of TB-infected cattle herds prior to and following proactive badger removal

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**Epidemiology and Infection 139, 1220-1229 (2011)**

Bovine tuberculosis (TB) is primarily a disease of cattle. In both Ireland and the UK, badgers (*Meles meles*) are an important wildlife reservoir of infection. This paper examined the hypothesis that TB is spatially correlated in cattle herds, established the range of correlation and the effect, if any, of proactive badger removal on this. We also re-analysed data from the Four Area Project in Ireland, a large-scale intervention study aimed at assessing the effect of proactive badger culling on bovine TB incidence in cattle herds, taking possible spatial correlation into account. We established that infected herds are spatially correlated (the scale of spatial correlation is presented), but at a scale that varies with time and in different areas. Spatial correlation persists following proactive badger removal.

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## Tuberculosis in cattle herds are sentinels for *Mycobacterium bovis* infection in European badgers (*Meles meles*): the Irish Greenfield Study

Murphy, D.<sup>1</sup>, Gormley, E.<sup>1</sup>, Collins, D.M.<sup>2</sup>, McGrath, G.<sup>2</sup>, Sovsic, E.<sup>3</sup>, Costello, E.<sup>3</sup>, Corner, L.A.L.<sup>1</sup>

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### Veterinary Microbiology 151, 120-125 (2011)

In Ireland, badgers are removed in response to tuberculosis (TB) breakdowns in cattle herds (focal culling). Prevalence studies, conducted using a detailed post mortem and bacteriological examination, showed that 36-50% of badgers were infected with *Mycobacterium bovis*. Focal culling forms part of the medium term national strategy for the control of bovine TB in cattle and is based on the premise that badgers in areas with herd breakdowns have a higher prevalence of infection than the badger population at large. However, the hypothesis that cattle can be used as sentinels for infection in the badger population has never been formally tested. In this study we tested the hypothesis by determining the infection prevalence in badgers in areas where there had been historically, a consistently low prevalence of infection in cattle. Low cattle TB prevalence areas were defined as those herds with  $\leq 2$  standard reactors in the annual round of skin testing over the preceding 5 years (Greenfield sites). Using GIS, and adjusting for variation in land use, previous culling and cattle density, 198 Greenfield sites were identified and surveyed, and 138 areas with badger setts or signs of badger activity were identified. A single badger was removed from 87 sites and all were examined using detailed post mortem and bacteriological procedures. A prevalence of *M. bovis* infection of 14.9% was found in the Greenfield site badgers. This prevalence was significantly lower ( $P < 0.001$ ) than in badgers removed during focal culling (36.6%). The results validate the use of cattle as sentinels for TB in badgers and support the medium term national strategy for the control of bovine TB. The geographic variation in *M. bovis* infection prevalence in the Irish badger populations will be used when devising strategies for the incorporation of badger vaccination into the long term bovine TB control programme.

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## Mycobacterial infections in multiple species: Implications for diagnosis and control

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### The Veterinary Journal (in press)

This paper discusses the significance of the ever-increasing numbers of different pathogenic mycobacterial species affecting both domestic and wild animals. Through advances in diagnostics and increased epidemiological investigations, it should be possible to define the reservoir status of each animal host and to establish the significance in terms of infection risk.

*Adapted from The Veterinary Journal 191, Corner, L.A.L., Gormley, E., Mycobacterial infections in multiple species: Implications for diagnosis and control, 141-142, Copyright 2012, with permission from Elsevier.*

## *Mycobacterium bovis* infection in the Eurasian badger (*Meles meles*): the disease, pathogenesis, epidemiology and control

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**Journal of Comparative Pathology 144, 1-24 (2011)**

Eurasian badgers (*Meles meles*) are an important wildlife reservoir of tuberculosis (*Mycobacterium bovis*) infection in Ireland and the United Kingdom. As part of national programmes to control tuberculosis in livestock, considerable effort has been devoted to studying the disease in badgers and this has led to a rapid increase in our knowledge of tuberculosis in this host. Tuberculosis in badgers is a chronic infection and in a naturally-infected population the severity of disease can vary widely, from latent infection (infection without clinical signs and no visible lesions) to severe disease with generalized pathology. The high prevalence of pulmonary infection strongly supports the lungs as the principal site of primary infection and that inhalation of infectious aerosol particles is the principal mode of transmission. However, other routes, including transmission via infected bite wounds, are known to occur. The ante mortem diagnosis of infection is difficult to achieve, as clinical examination and immunological and bacteriological examination of clinical samples are insensitive diagnostic procedures. Because infection in the majority of badgers is latent, the gross post mortem diagnosis is also insensitive. A definitive diagnosis can only be made by the isolation of *M. bovis*. However, to gain a high level of sensitivity in the bacteriological examination, a large number of tissues from each badger must be cultured and sensitive culture methods employed. The transmission and maintenance of *M. bovis* in badger populations are complex processes where many factors influence within-population prevalence and rates of transmission. Badger social structures and the longevity of infected animals make them an ideal maintenance host for *M. bovis* infection. Badgers are directly implicated in the transmission of infection to cattle and the inability to eradicate the disease from cattle is, in part, a consequence of the interactions between the two species. A detailed understanding and knowledge of the epidemiology and pathogenesis of the disease are recognized as fundamental for devising new strategies to control infection with a view to limiting interspecies transmission. Vaccination, in spite of formidable challenges, is seen as the best long-term strategy option and studies with captive badgers have shown that vaccination with *M. bovis* Bacillus Calmette-Guérin (BCG) induces protection when delivered by a variety of routes. Continued research is required to develop effective technologies to control the disease both in badgers and cattle. A combination of strategies, which employ the optimal use and targeting of resources, is likely to make a significant contribution towards eradication of the disease.

*Adapted from Journal of Comparative Pathology, 144, Corner, L.A.L., Murphy, D., Gormley, E., Mycobacterium bovis infection in the Eurasian badger (Meles meles): the disease, pathogenesis, epidemiology and control, 1-24, Copyright 2011, with permission from Elsevier.*

## The prevalence and distribution of *Mycobacterium bovis* infection in European badgers (*Meles meles*) as determined by enhanced post mortem examination and bacteriological culture

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**Research in Veterinary Science 88, 1-5 (2010)**

The accurate diagnosis of *Mycobacterium bovis* infection in badgers is key to understanding the epidemiology of tuberculosis in this species and has significant implications for devising strategies to limit spread of the disease. In this study, badgers ( $n=215$ ) in the Republic of Ireland were examined post mortem and tissues were collected from a range of anatomical locations and pooled into groups for bacterial culture of *M. bovis*. By assessing confirmed gross visible lesions

(VL) alone, infection was detected in 12.1% of badgers. However, by including the results of all culture positive pooled samples, the overall infection prevalence increased significantly to 36.3%. Two-thirds (66.7%) of infected animals had no visible lesions (NVL). While the thoracic cavity (lungs and pulmonary lymph nodes) was found to be the most common site of infection, in a proportion of animals infection was absent from the lungs and draining lymph nodes and was confined to the lymph nodes of the carcase or the head. This may indicate an early extrapulmonary dissemination of infection or alternatively, in the case of the head lymph nodes, a secondary pathogenic pathway involving the lymphoid tissues of the upper respiratory tract (URT).

*Reprinted from Research in Veterinary Science, 88, Murphy, D., Gormley, E., Costello, E., O'Meara, D., Corner, L.A.L., The prevalence and distribution of Mycobacterium bovis infection in European badgers (Meles meles) as determined by enhanced post mortem examination and bacteriological culture, 1-5, Copyright 2010, with permission from Elsevier.*

## Infection control strategies

### Badger removal

#### A comparison of the effectiveness of badger removal programs: Wildlife Administration Unit vs. pre-2004 licenced capture

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<sup>1</sup> UCD CVERA

In response to severe outbreaks of tuberculosis in cattle, an epidemiological investigation is carried out to establish the source of infection. If badgers are implicated, badger removal is conducted under licence by the Department of Agriculture, Food and the Marine. From the late 1980's until 2004, the badger removal program comprised of the removal of badgers as a single event from setts found within 1 kilometre of the breakdown herd. Since 2004, the method of badger removal changed from this single removal event to a continued removal of badgers from setts (main setts 1.5 kilometres and other setts 2km from the breakdown herd). This study aims to compare the effectiveness of these two badger removal approaches by examining their comparative survival times following the initial badger removals. Severity of future breakdowns could also be used as a measure of determining effectiveness. The study could be expanded to include the effect on neighbouring farms and/or farms up to a defined distance.

#### Risk-based badger removal and the subsequent herd breakdown rate for Bovine Tuberculosis in Co. Monaghan, Ireland

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<sup>1</sup> UCD CVERA, <sup>2</sup> DAFM

For the purposes of this study, a herd is classed as an "index herd" where, following an ER76 epidemiological investigation into the source of a bTB breakdown, approval is granted for local badger removal. This study aims to assess the effect of local badger removal on the risk of a next herd bTB breakdown among neighbouring herds surrounding an index herd. To achieve this, we will conduct a survival analysis with the outcome being time to next breakdown for herds surrounding setts from which badgers were removed under the Wildlife Unit program. Herd exposure is measured as the proportion of a herd(s) land area within 1km of any sett removal. We will test the hypothesis that the hazard of bTB for herds with a high percentage of land area within 1km of sett removal do not differ from herds with a low percentage of land area within 1km of sett removal.

## Progress towards a badger vaccine

### Badger tuberculosis vaccine

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#### *a. Vaccine development (studies with captive badgers)*

The first phase of research into BCG vaccination against tuberculosis in captive badgers, an integrated series of seven experiments and associated studies that commenced in 2001, has been completed. These studies have established as proof of principle that vaccination of badgers with BCG, when delivered by a variety of routes, can protect badgers against tuberculosis. The BCG vaccine has been chosen for use based on its availability, low production cost and much experience of its application in domestic and wild animals, and humans. An oral vaccine developed by Dr. Frank Aldwell, Otago University, New Zealand, has been shown to work in captive badgers and paves the way for development of oral delivery systems for use in wild badgers. In our most recent study, we have shown that the oral vaccine is effective against challenge with a low dose of *M. bovis*, at levels likely to be encountered under conditions of natural transmission. The data has given us a much better understanding of how the vaccine is likely to perform under natural conditions and will facilitate the development of strategies to deliver the vaccine to badger populations. As part of these studies, and working closely with colleagues at AHVLA (Weybridge UK), we helped develop and assess a range of *in vitro* diagnostic assays based on the immunological responses of badgers to challenge with *M. bovis*.

#### *b. The vaccine field trial*

The success of the captive badger studies has paved the way for the testing of oral BCG vaccine in a large-scale field trial. The BCG vaccine field trial has two principal objectives. These are to validate the results of captive badger studies and show that BCG vaccine is protective in naturally exposed wild badgers, and to estimate vaccine efficacy under field conditions. The aim of the field trial is to estimate the efficacy of BCG vaccine and to demonstrate protection in a wild badger population by comparing prevalence of tuberculosis in vaccinated and non-vaccinated badgers after three years. A secondary outcome of the field trial will be to measure the effect of BCG vaccine in badgers with pre-existing *M. bovis* infection. In addition to providing a measurement of protection and an estimate of vaccine efficacy, the field trial will provide a practical basis for understanding the logistics of oral vaccine delivery to wild badger populations.

The first capture sweep commenced in September 2009 and, to date, five capture sweeps of the vaccine field trial has been completed. The trial area of approx. 755 square kilometres is divided into three zones (Figure 1) each with a different level of vaccine coverage. Vaccine and placebo are blind coded and field staff are unaware of the vaccine status of the badgers they are treating. Badgers captured in the middle zone are randomly assigned vaccine or placebo on approximately a 50:50 basis, 100% of badgers captured in the north and south will receive either vaccine or placebo therefore there will be a gradient of vaccine cover from 0% to 50% to 100% vaccination from north to south or south to north.

- The entire trial area has been trapped for the fifth time over Autumn/Winter 2011
- During the fifth sweep of the trial area, badgers were captured and examined on 250 occasions (Table 1)
- The total treatments to date across the three zones since start of field trial is shown in Table 2



Figure 1 – Outline of vaccine trial area and divisions into three zones

Table 1. Age and sex of the badgers examined (n = 250) in sweep 5 of the vaccine trial

	Cub	Juvenile	Adult	Old	Total
Female	0	38	69	19	126
Male	0	34	82	8	124
Total	0	72	151	27	250

Table 2. Treatment to date in the three zones of the trial area\*

	1*	2*	Total**
Zone A	0	408	408
Zone B	99	108	207
Zone C	369	1	370
Total	468	517	985

\* Badgers receive either vaccine or placebo, blind-coded 1 & 2

\*\* Total includes 208 badgers that were revaccinated in sweep 3, 4 and 5

At the end of the 3 year study period (Aug 2012), the trial site will be depopulated and all badgers will be examined for tuberculosis by detailed post mortem examination that will include an examination for visible lesions, histologic lesions, and mycobacteriology. The isolation of *M. bovis* from post mortem or clinical samples (wound exudates or tracheal swabs) will be used to define a case of tuberculosis. The results and experience gained from the field trial will facilitate the development of strategies for introduction of vaccination into the national program.

### c. Development of badger immunodiagnostics

We have continued to develop and assess a range of immunodiagnostic tests that will be required for tuberculosis surveillance in badger populations to monitor the effect of vaccination. This work, carried out in collaboration with AHVLA Weybridge and with Enfer group, is focused on developing cell-mediated immune and antibody based diagnostic tests for badgers. To date, we have established that the sensitivity of all the immunodiagnostic assays improves as the disease severity increases and that this is more pronounced with the serological based assays. The serology tests are being applied to the field trial blood samples and will assist in the validation of the tests on naturally infected badgers.



## Trial design to estimate the effect of vaccination on tuberculosis incidence in badgers

Aznar, I.<sup>1</sup>, McGrath, G.<sup>1</sup>, Murphy, D.<sup>2</sup>, Corner, L.A.L.<sup>3</sup>, Gormley, E.<sup>3</sup>, Frankena, K.<sup>4</sup>, More, S.J.<sup>1,3</sup>, Martin, W.<sup>5</sup>, O'Keeffe, J.<sup>1,6</sup>, De Jong, M.C.M.<sup>4</sup>

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### Veterinary Microbiology 151, 104-111 (2011)

The principal wildlife reservoir of *Mycobacterium bovis* in Ireland is the European badger. Studies in the Republic of Ireland (RoI) have shown that badgers culled in association with cattle herd tuberculosis breakdowns (focal culling) have a higher prevalence of infection than the badger population at large. This observation is one rationale for the medium term national strategy of focal badger culling. A vaccination strategy for the control of bovine tuberculosis (bTB) in badgers is a preferred long-term option. The Bacillus Calmette-Guérin (BCG) vaccine has been shown to decrease disease severity in captive badgers under controlled conditions. As the vaccine has been tested in a controlled environment with precise information on infection pressure, it cannot be assumed *a priori* that the effects of vaccination are similar in the wild, where other environmental and/or ecological factors prevail. For this reason we have designed a vaccine field trial to assess the impact of vaccination on the incidence of TB infection in a wild badger population. The selected study area for the vaccine trial (approximately 755 square kilometers) is divided into three zones each of which has similar characteristics in terms of size, number of main badger setts, cattle herds, cattle and land classification type. Three vaccination levels (100%, 50% and 0%) will be allocated to the three zones in a way that a gradient of vaccination coverage North to South is achieved. The middle zone (zone B) will be vaccinated at a 50% coverage but zone A and C will be randomly allocated with 100% or 0% vaccination coverage. Vaccination within zone B will be done randomly at individual badger level. The objective of this paper is to describe the design of a field tuberculosis vaccination trial for badgers, the epidemiological methods that were used to design the trial and the subsequent data analysis. The analysis will enable us to quantify the magnitude of the observed vaccination effect on *M. bovis* transmission in badgers under field conditions and to improve our knowledge of the biological effects of vaccination on susceptibility and infectiousness.

*Reprinted from Veterinary Microbiology, 151, Aznar, I., McGrath, G., Murphy, D., Corner, L.A.L., Gormley, E., Frankena, K., More, S.J., Martin, W., O'Keeffe, J., De Jong, M.C.M., Trial design to estimate the effect of vaccination on tuberculosis incidence in badgers, 104-111, Copyright 2011, with permission from Elsevier.*

## Using simulation to estimate the power of a badger vaccine trial against *Mycobacterium bovis* in badgers

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The aim of this study was to estimate the power of a badger vaccine field trial designed in the Republic of Ireland to help in the control of bovine tuberculosis using simulation techniques. The effects of sample size (recapture percentage), sensitivity and specificity of the diagnostic test, transmission rate between unvaccinated badgers, Vaccine Efficacy for Susceptibility (VES) and Vaccine Efficacy for Infectiousness (VEI), on study power were determined. Sample size had a small effect on power. Study power increased with increasing transmission rate between non-vaccinated badgers. Changes in VES had a higher impact on power than changes in VEI. However, the largest effect on study power was seen by changes in the specificity of the diagnostic test. The effect of changes in sensitivity on study power was much lower. Therefore, it is critical that the diagnostic test used in the badger vaccine trial is optimized to maximise test specificity.

## Infection dynamics and effective control strategies of tuberculosis in badgers and cattle of Ireland

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The main objective is to assess the impact of interventions on bovine tuberculosis (bTB) prevalence in cattle and badgers; for this a mathematical model of bTB transmission that describes the disease in cattle and badgers in the Republic of Ireland will be developed. The hypothesis is that control, with the ultimate goal of eradication, cannot be achieved until badger-to-cattle transmission is effectively addressed. Data from ongoing field trials (particularly data related to efficacy of the badger vaccine) and data from previous research work will be utilized to inform parameter estimation. Experimental work (the exact nature of which will depend on identified gaps in the knowledge required for parameter estimation) will also be carried out.

## Optimisation of a multiple antigen ELISA test to be used in a badger vaccine trial

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The main objective of this study was to optimize a diagnostic test for *M. bovis* infection in live badgers trapped during the badger vaccine trial in Ireland using a multiplex chemiluminiscent assay (Enfer Scientific). A total of 215 blood samples (including 200 blood samples used by Whelan *et al.*, 2009) were tested against a panel of 8 *M. bovis* antigens. The optimization was done while keeping specificity at 99.99% to allow for a reasonable power (60-80%) to be obtained in the vaccine trial. We also aimed to explore the effects of vaccination on test characteristics and review the implications for analysis of the data obtained from the Kilkenny badger vaccine trial.



## Bacillus Calmette-Guérin vaccination reduces the severity and progression of tuberculosis in badgers

Chambers, M.A.<sup>1</sup>, Rogers, F.<sup>1,2</sup>, Delahay, R.J.<sup>2</sup>, Lesellier, S.<sup>1</sup>, Ashford, R.<sup>1</sup>, Dalley, D.<sup>1</sup>, Gowtage, S.<sup>1</sup>, Davé, D.<sup>1</sup>, Palmer, S.<sup>1</sup>, Brewer, J.<sup>1</sup>, Crawshaw, T.<sup>1</sup>, Clifton-Hadley, R.<sup>1</sup>, Carter, S.<sup>2</sup>, Cheeseman, C.<sup>2</sup>, Hanks, C.<sup>2</sup>, Murray, A.<sup>2</sup>, Palphramand, K.<sup>2</sup>, Pietravalle, S.<sup>2</sup>, Smith, G.C.<sup>2</sup>, Tomlinson, A.<sup>2</sup>, Walker, N.J.<sup>2</sup>, Wilson, G.J.<sup>2</sup>, Corner, L.A.L.<sup>3</sup>, Rushton, S.P.<sup>4</sup>, Shirley, M.D.F.<sup>4</sup>, Gettinby, G.<sup>5</sup>, McDonald, R.A.<sup>2</sup>, Hewinson, R.G.<sup>1</sup>

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### Proceedings of the Royal Society B 278, 1913-1920 (2011)

Control of bovine tuberculosis (TB) in cattle has proven particularly challenging where reservoirs of infection exist in wildlife populations. In Britain and Ireland, control is hampered by a reservoir of infection in Eurasian badgers (*Meles meles*). Badger culling has positive and negative effects on bovine TB in cattle and is difficult, costly and controversial. Here we show that *Bacillus Calmette-Guérin* (BCG) vaccination of captive badgers reduced the progression, severity and excretion of *Mycobacterium bovis* infection after experimental challenge. In a clinical field study, BCG vaccination of free-living badgers reduced the incidence of positive serological test results by 73.8 per cent. In common with other species, BCG did not appear to prevent infection of badgers subjected to experimental challenge, but did significantly reduce the overall disease burden. BCG vaccination of badgers could comprise an important component of a comprehensive programme of measures to control bovine TB in cattle.

*Reprinted from Proceedings of the Royal Society B, 278, Chambers, M.A., Rogers, F., Delahay, R.J., Lesellier, S., Ashford, R., Dalley, D., Gowtage, S., Davé, D., Palmer, S., Brewer, J., Crawshaw, T., Clifton-Hadley, R., Carter, S., Cheeseman, C., Hanks, C., Murray, A., Palphramand, K., Pietravalle, S., Smith, G.C., Tomlinson, A., Walker, N.J., Wilson, G.J., Corner, L.A.L., Rushton, S.P., Shirley, M.D.F., Gettinby, G., McDonald, R.A., Hewinson, R.G., Bacillus Calmette-Guérin vaccination reduces the severity and progression of tuberculosis in badgers, 1913-1920, Copyright 2011, with permission from The Royal Society.*

## Oral vaccination of badgers (*Meles meles*) with BCG and protective immunity against endobronchial challenge with *Mycobacterium bovis*

Corner, L.A.L.<sup>1</sup>, Costello, E.<sup>2</sup>, O'Meara, D.<sup>2</sup>, Lesellier, S.<sup>1,5</sup>, Aldwell, F.E.<sup>3</sup>, Singh, M.<sup>4</sup>, Hewinson, R.G.<sup>5</sup>, Chambers, M.A.<sup>5</sup>, Gormley, E.<sup>1</sup>

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### Vaccine 28, 6265-6272 (2010)

Eurasian badgers (*Meles meles*) are a reservoir host of *Mycobacterium bovis* and are implicated in the transmission of tuberculosis to cattle in Ireland and Great Britain. The development of a vaccine for use in badgers is considered a key element of any long-term sustainable campaign to eradicate the disease from livestock in both countries. The aim of this study was to investigate the protective response of badgers vaccinated orally with Bacille Calmette-Guérin (BCG) encapsulated in a lipid formulation, followed by experimental challenge with *M. bovis*. A group of badgers was vaccinated by inoculating the BCG-lipid mixture containing approximately 10<sup>8</sup> colony forming units (cfu) of BCG into the oesophagus. The control group was sham inoculated with the lipid formulation only. Thirteen weeks after vaccination

all the badgers were challenged with approximately  $10^4$  cfu of *M. bovis* delivered by endobronchial inoculation. Blood samples were taken throughout the study and the cell mediated immune (CMI) responses in peripheral blood were monitored by the IFN-gamma ELISA and ELISPOT assay. At 17 weeks after infection all the badgers were examined post mortem to assess the pathological and bacteriological responses to challenge. All badgers in both groups were found to be infected. However, a significant protective effect of BCG vaccination was measured as a decrease in the number and severity of gross lesions, lower bacterial load in the lungs, and fewer sites of infection. The analysis of immune responses showed that vaccination with BCG did not generate any detectable CMI immunological responses, however the levels of the responses increased in both groups following *M. bovis* infection. The results of the study showed that vaccination with oral BCG in the lipid formulation generated a protective effect in the badgers.

*Reprinted from Vaccine, 28, Corner, L.A.L., Costello, E., O'Meara, D., Lesellier, S., Aldwell, F.E., Singh, M., Hewinson, R.G., Chambers, M.A., Gormley, E., Oral vaccination of badgers (Meles meles) with BCG and protective immunity against endobronchial challenge with Mycobacterium bovis, 6265-6272, Copyright 2010, with permission from Elsevier.*

## Control of tuberculosis in badgers by vaccination: Where next?

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**The Veterinary Journal 189, 239-241 (2011)**

With the development of an oral vaccine for use in badgers continuing, this paper examines a range of strategic options available for vaccination that might be employed to control and eradicate TB in badgers, and the beneficial impact this will have in cattle herds.

*Adapted from The Veterinary Journal, 189, Gormley, E., Corner, L.A.L., Control of tuberculosis in badgers by vaccination: Where next?, 239-241, Copyright 2011, with permission from Elsevier.*

## Evaluation of attractant flavours for use in oral vaccine baits for badgers (*Meles meles*)

Kelly, D.J.<sup>1</sup>, Corner, L.A.L.<sup>2</sup>, Gormley, E.<sup>2</sup>, Murphy, D.<sup>2</sup>, Costello, E.<sup>3</sup>, Aldwell, F.E.<sup>4</sup>, Marples, N.M.<sup>1</sup>

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**European Journal of Wildlife Research 57, 767-774 (2011)**

European badgers (*Meles meles*) are a wildlife reservoir for *Mycobacterium bovis* infection (tuberculosis) in Ireland and the UK and are implicated in the transmission of infection to livestock. Vaccination of badgers with the human BCG vaccine (Bacille Calmette Guérin) is considered as an important strategy to reduce the burden of disease in this species, and a pragmatic approach is likely to involve oral vaccination. In this study, we evaluated nine different flavours for use as attractants in a prototype oral vaccine bait for European badgers (*M. meles*): aniseed, apple, cocoa powder, carob powder, curry, fish, garlic, peanut and strawberry. The bait matrix was composed of a natural lipid formulation, developed as a vehicle for oral vaccination against tuberculosis in wildlife. A 'food for work' paradigm was employed during the trials to ensure the animals were actively seeking the baits. The trials showed carob and cocoa powders were equally attractive and more attractive than any of the other candidates. Carob and cocoa show potential as bait attractants for badgers and might form part of a novel vaccine delivery system.

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## The development of a bait delivery system for oral vaccination of badgers against tuberculosis

Kelly, D.J.<sup>1</sup>, Gormley, E.<sup>2</sup>, Corner, L.A.L.<sup>2</sup>, Marples, N.M.<sup>1</sup>

<sup>1</sup> TCD School of Natural Sciences, <sup>2</sup> UCD School of Veterinary Medicine

This work has identified the potential of carob as an attractant for use with oral vaccine baits and has established the importance of pre-feeding when introducing "flapjack" baits to "naïve" Irish badger populations. We have found that pre-feeding was most effective when the trial baits were most similar to the pre-fed baits, and that packaged baits can be ignored if they are not coated in an attractant. The results also suggest that burying baits may stimulate natural feeding behaviours. This could imply that buried baits may be more attractive to badgers than baits placed in sett entrances, despite the latter being more easily available. The development of a bait for oral vaccination is continuing.



## Protection of Eurasian badgers (*Meles meles*) from tuberculosis after intra-muscular vaccination with different doses of BCG

Lesellier, S.<sup>1</sup>, Palmer, S.<sup>1</sup>, Gowtage-Sequiera, S.<sup>1</sup>, Ashford, R.<sup>1</sup>, Dalley, D.<sup>1</sup>, Davé, D.<sup>1</sup>, Weyer, U.<sup>1</sup>, Salguero, F.J.<sup>1</sup>, Nuñez, A.<sup>1</sup>, Crawshaw, T.<sup>2</sup>, Corner, L.A.L.<sup>3</sup>, Hewinson, R.G.<sup>1</sup>, Chambers, M.A.<sup>1</sup>

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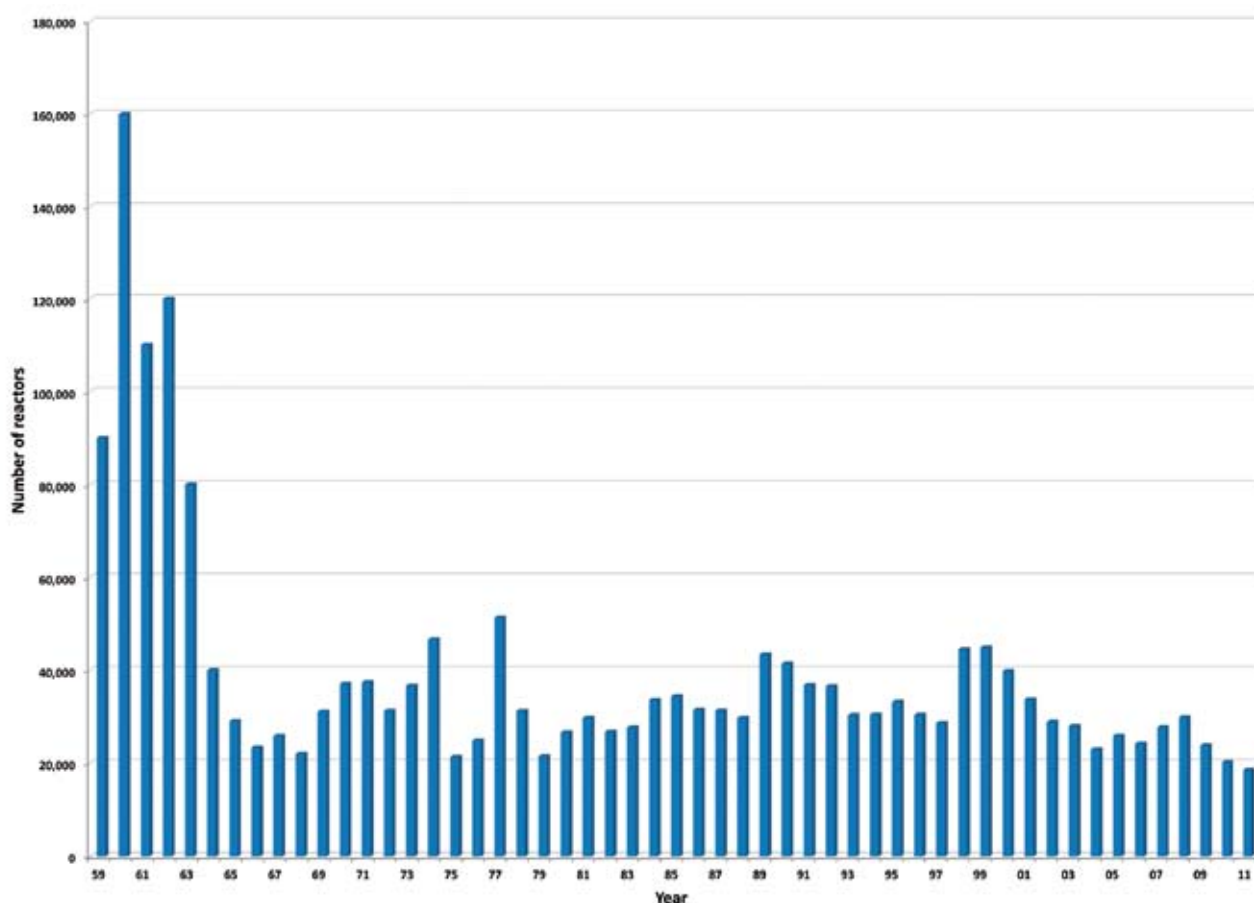
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### Vaccine 29, 3782–3790 (2011)

*Mycobacterium bovis* infection is widespread in Eurasian badger (*Meles meles*) populations in Great Britain and the Republic of Ireland where they act as a wildlife reservoir of infection for cattle. Removal of infected badgers can significantly reduce the incidence of bovine tuberculosis (TB) in local cattle herds. However, control measures based on culling of native wildlife are contentious and may even be detrimental to disease control. Vaccinating badgers with Bacillus Calmette-Guérin (BCG) has been shown to be efficacious against experimentally induced TB of badgers when administered subcutaneously and orally. Vaccination may be an alternative or complementary strategy to other disease control measures. As the subcutaneous route is impractical for vaccinating wild badgers and an oral vaccine bait formulation is currently unavailable, we evaluated the intramuscular (IM) route of BCG administration. It has been demonstrated that the IM route is safe in badgers. IM administration has the practical advantage of being relatively easy to perform on trapped wild badgers without recourse to chemical immobilisation. We report the evaluation of the efficacy of IM administration of BCG Danish strain 1331 at two different doses: the dose prescribed for adult humans ( $2\text{--}8 \times 10^5$  colony forming units) and a 10-fold higher dose. Vaccination generated a dose-dependent cell-mediated immune response characterised by the production of interferon- $\gamma$  (IFN $\gamma$ ) and protection against endobronchial challenge with virulent *M. bovis*. Protection, expressed in terms of a significant reduction in the severity of disease, the number of tissues containing acid-fast bacilli, and reduced bacterial excretion was statistically significant with the higher dose only.

Reprinted from Vaccine, 29, Lesellier, S., Palmer, S., Gowtage-Sequiera, S., Ashford, R., Dalley, D., Davé, D., Weyer, U., Salguero, F.J., Nuñez, A., Crawshaw, T., Corner, L.A.L., Hewinson, R.G., Chambers, M.A., Protection of Eurasian badgers (*Meles meles*) from tuberculosis after intra-muscular vaccination with different doses of BCG, 3782–3790, Copyright 2011, with permission from Elsevier.

## The national programme



*The number of TB reactors detected in Ireland each year between 1959 and 2011.*

### Progress in tuberculosis eradication in Ireland

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<sup>1</sup> DAFM

#### **Veterinary Microbiology 151, 160-169 (2011)**

Ireland ran a conventional test and slaughter Bovine Tuberculosis eradication programme from 1954 until 1988. This programme fulfilled our trading requirements but failed to eradicate TB. At this point a major initiative, ERAD, was launched targeted with reducing the disease levels by half within a four-year period and devising the strategy and supports necessary to achieve final eradication. The lessons learned at that time have informed Ireland's eradication programme ever since. Eradication was not possible without developing solutions to address the wildlife disease reservoir and other identified constraints. Since 1992 the programme objectives have been restated. It is now effectively an interim control programme where significant resources have been invested in research and development aimed at overcoming the identified constraints to eradication. Policy is informed by science and debate among stakeholders is generally knowledgeable and balanced. This paper outlines developments in recent years and sets out our expectations for progress in the period ahead.

*Reprinted from Veterinary Microbiology, 151, Sheridan, M., Progress in tuberculosis eradication in Ireland, 160-169, Copyright 2011, with permission from Elsevier.*

## Perspectives on the history of bovine TB and the role of tuberculin in bovine TB eradication

Good, M.<sup>1</sup>, Duignan, A.<sup>1</sup>

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**Veterinary Medicine International, article ID 410470 (2011)**

Tuberculosis remains a significant disease of animals and humans worldwide. Bovine tuberculosis is caused by *Mycobacteria* with an extremely wide host range and serious, although currently probably underdiagnosed, zoonotic potential. Where bovine tuberculosis controls are effective, human zoonotic TB, due to *Mycobacterium bovis* or *M. caprae*, is uncommon and clinical cases are infrequent in cattle. Therefore, the control and ultimate eradication of bovine tuberculosis is desirable. Tuberculin tests are the primary screening tool used in bovine eradication. The choice of tuberculin test is dependent on the environment in which it is to be used. Tuberculin potency is critical to test performance, and the accurate determination of potency is therefore particularly important. The design of a control or eradication programme should take into consideration the fundamental scientific knowledge, the epidemiological profile of disease, the experience of other eradication programmes, and the presence, in the same ecosystem, of maintenance hosts, in which infection is self-sustaining and which are capable of transmitting infection. A control or eradication programme will necessarily require modification as it progresses and must be under constant review to identify the optimal desirable goals, the efficacy of policy, and constraints to progress.

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## Management of the Irish TB eradication programme: the development and application of new measures of performance

Higgins, I.M.<sup>1</sup>, Williams, D.H.<sup>2</sup>, More, S.J.<sup>1,3</sup>

<sup>1</sup> UCD CVERA, <sup>2</sup> UCD School of Mathematical Sciences, <sup>3</sup> UCD School of Veterinary Medicine

Performance management in national TB programmes has not been straight-forward. There is a broad range of reasons for this, including infection epidemiology, test interpretation and data collection and management. As one example of the complexity involved, although TB-related data in Ireland are collected at the level of the event (test, breakdown), some relate to the animal (which is nested within herd) and some to the herd. In Ireland, national progress is currently measured using the number of reactors per 1,000 tests (APT), which is computationally simple, however, there are a number of difficulties with this approach. This paper describes work to define performance measures to assist with ongoing review of the Irish programme, to create automated methods to enable ongoing measurement, and to review progress. A range of performance measures, linked to defined periods of herd trading restriction following indications of *M. bovis* infection, have been developed to separately assess the effectiveness of surveillance (to detect new cases: herd disease incidence, percentage of herds remaining disease free) and control (to clear infection following case detection: restriction duration, number of reactors per restriction, % single reactor breakdowns, % 3rd/4th reactor retests, inter-episode interval, repeat restrictions). Programming was conducted using SAS® (SAS Institute Inc., Cary, NC, USA) with extensive use of SAS® macro programming, using TB herd summary results from the national Animal Health Computer System (AHCS) as data inputs. The final set of programmes can be run without intervention. The surveillance and control measures have been calculated for all active Irish herds, and summarized by District Veterinary Office (unit of programme management), production type, herd size and past disease history. In contrast to current methods, these herd-level measures effectively

partition activities in Ireland relating to detection of new cases (surveillance) and the resolution of cases following detection (control). This information, provided on an ongoing and timely basis will be of benefit to national and regional programme decision-makers.

## TB in Ireland - visualising the current picture

McGrath, G.<sup>1</sup>

<sup>1</sup> UCD CVERA

The current methodologies used to visualise the prevalence of tuberculosis in Ireland include thematic mapping (positive animal tests per thousand tests per district electoral division) and density maps (kernel densities of positive animals/population). This project will investigate possible new ways of displaying the changes in tuberculosis prevalence over time to provide a better visualisation of the recent decrease in reactor numbers. A methodology is required that is more sensitive to representing proportionally small changes. The proposed method will involve assigning herd level data to a uniform hexagonal grid. A measure of mean prevalence will be calculated for a defined period of time. Each year will then be displayed as a deviation above or below this mean level thus showing the relative temporal trend in prevalence for each unit through time.

## An evaluation of the Irish Single Reactor Breakdown Protocol for 2005 to 2008 inclusive and its potential application as a monitor of tuberculin test performance

Good, M.<sup>1</sup>, Duignan, A.<sup>1</sup>

<sup>1</sup> DAFM

### **Veterinary Microbiology 151, 85-90 (2011)**

Under the Irish Bovine Tuberculosis (bTB) Eradication Programme all herds are subjected to at least one test *per annum*. The Single Intradermal Comparative Tuberculin Test (SICTT) is used in Ireland for the detection of cattle infected with *Mycobacterium bovis*. There have been concerns regarding the specificity of the SICTT, notably by farmers, and particularly in herds where the detection of a single positive animal in the absence of an obvious source of (bTB) infection could be perceived as a “false” positive. To address this issue the so-called ‘Singleton Protocol’ was established as part of the Irish bTB eradication programme. This protocol allows for the early restoration of free trading status to herds where a single positive animal was detected and where the herd was not confirmed as infected with *M. bovis* by epidemiological investigation, by post mortem, by laboratory examination, or by further test. This paper presents data from the 2005 to 2008, inclusive, bTB programmes on the number of herds that were assessed, which qualified for inclusion under the ‘Singleton Protocol’ and the outcome for qualifying herds up to and including having status restored early as a consequence of inclusion in that programme. The outcome of this protocol reaffirms the reliability of the SICTT at current levels of infection. Furthermore it is advocated that the ‘Singleton Protocol’ be continued as a monitor of herds in which a single positive animal is disclosed, and as overall infection levels of bTB fall the outcome may be used as one means to assess progress towards bTB eradication in Ireland.

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## Singleton area risk: Can areas of temporo-spatial clustering in singleton reactors be defined?

McGrath, G.<sup>1</sup>

<sup>1</sup> UCD CVERA

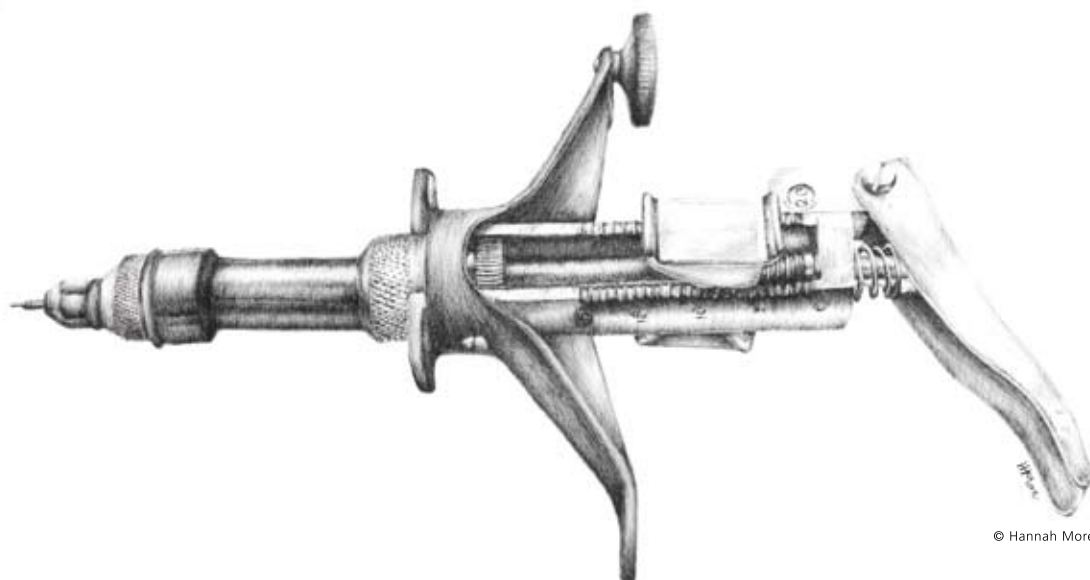
There is evidence that non-specific infection or singleton reactors occur as a result of environmental conditions found on farms close to bog, reclaimed wetland and lakes. If this is the case, they should cluster in space and time. We would expect there to be a spatial consistency which should be discernible. The objective of this study is to attempt to identify if this spatial/temporal clustering of singleton reactors exists. If there is a discernible effect, areas of persistent high singleton risk could be defined and assigned back to all herds falling within these areas providing veterinary inspectors with an additional layer of information to aid in deciding how to interpret and manage herds with singleton reactors.

## Evaluation of single reactor bovine tuberculosis breakdowns, based on analysis of reactors slaughtered at an Irish export meat plant

Murray, D.<sup>1</sup>, Clegg, T.A.<sup>2</sup>, More, S.J.<sup>2, 3</sup>

<sup>1</sup> DAFM, <sup>2</sup> UCD CVERA, <sup>3</sup> UCD School of Veterinary Medicine

The 'Singleton Protocol' was adopted by the Irish Department of Agriculture, Food and the Marine (DAFM) in 1996 to address the incomplete specificity of the Single Intradermal Comparative Tuberculin Test (SICTT) used in Ireland for the detection of animals infected with bovine tuberculosis (bTB). The protocol allows the early restoration of disease-free status to herds with a single reactor breakdown, where the herd was not confirmed as infected with *Mycobacterium bovis* by epidemiological investigation, by post mortem examination or by further test. The current study examines the ability of the Singleton Protocol to identify false positive reactors, the subsequent herd reactor rate following single reactor removal and analyses the factors leading to a positive post mortem lesion outcome and a positive reactor retest result. Post mortem lesion results were obtained for 371 reactor animals from single reactor breakdowns that were killed at an export meat plant over a nineteen-month period. Epidemiological and test data for these animals and their herds were obtained from DAFM databases and analysed by univariate and multivariate statistical analysis. Singleton candidates had an 18.7% lower lesion rate than single animal breakdowns not meeting the singleton criteria. No significant difference was found between singletons and non-singletons in the subsequent reactor retest results. Skin thickness at the SICTT is the most significant determinant of a positive lesion result. The area bTB history was shown to be a significant variable in producing a positive reactor retest result.



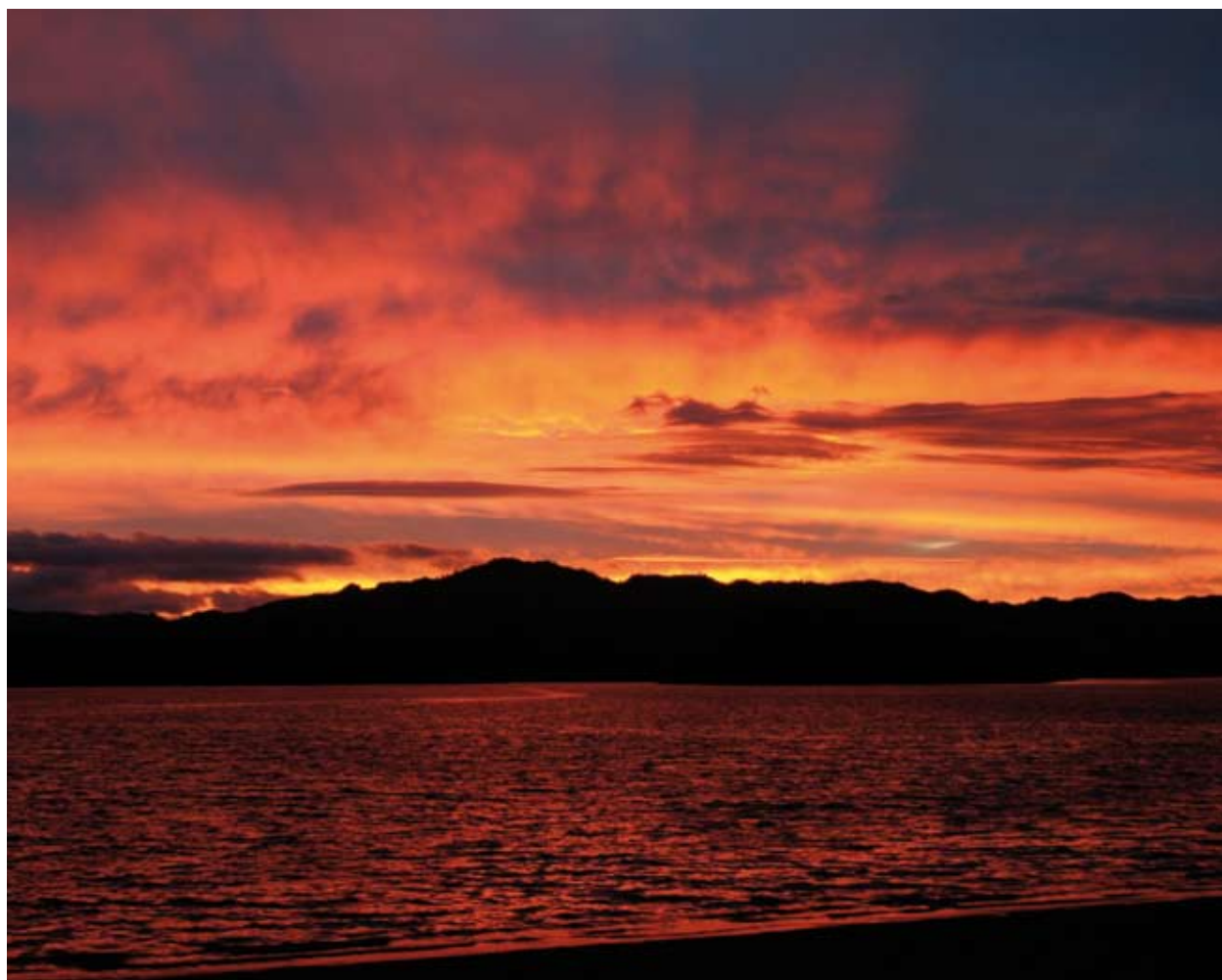
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## Bovine tuberculosis trends in the United Kingdom and Republic of Ireland, 1995 to 2010

Abernethy, D.A.<sup>1</sup>, Upton, P.<sup>2</sup>, Higgins, I.M.<sup>3</sup>, McGrath, G.<sup>3</sup>, Goodchild, T.<sup>2</sup>, Rolfe, S.<sup>4</sup>, Broughan, J.<sup>2</sup>, Downes, S.<sup>2</sup>, Clifton-Hadley, R.<sup>2</sup>, Menzies, F.<sup>1</sup>, de la Rua-Domenech, R.<sup>5</sup>, Blissit, M.<sup>6</sup>, Duignan, A.<sup>7</sup>, More, S.J.<sup>3,8</sup>

<sup>1</sup> Department of Agriculture and Rural Development, Belfast, Northern Ireland, <sup>2</sup> Animal Health and Veterinary Laboratories Agency, Weybridge, United Kingdom <sup>3</sup> UCD CVERA, <sup>4</sup> Office of the Chief Veterinary Officer, Welsh Assembly, Cardiff, Wales, <sup>5</sup> Department for Environment, Food and Rural Affairs, London, England, <sup>6</sup> Scottish Government, Edinburgh, Scotland, <sup>7</sup> DAFM, <sup>8</sup> UCD School of Veterinary Medicine

European legislation, principally 64/432 EEC and 78/52 EEC, forms the basis of national programmes and governs the surveillance and control measures applied by Member States. The United Kingdom includes the countries of Northern Ireland, England, Wales and Scotland. The latter three are collectively referred to as Great Britain while Northern Ireland is located with the Republic of Ireland on the island of Ireland, separated from Great Britain by the Irish Sea. Historically, bTB policy within the United Kingdom was determined separately for Great Britain and Northern Ireland, leading to differences in strategy and management. Devolution of responsibility for agriculture to the Welsh and Scottish administrations in 1998 further regionalised policy development. Thus, although programme measures were largely standardised through European legislation, some differences occurred, largely in response to specific risks or bTB prevalence. Comparing bTB trends provides an opportunity to learn from differing experiences and gain possible insight into factors that may promote or impede progress towards eradication. Such comparisons, however, are hampered by differences in measurement of disease parameters and summary statistics. This project will seek to identify and wherever possible, reduce these differences to allow direct comparison of trends between the jurisdictions.



*Photograph by E. Gormley.*



## Bovine tuberculosis in Europe from the perspective of an officially tuberculosis - free country: Trade, surveillance and diagnostics

Schiller, I.<sup>1</sup>, Waters, W.R.<sup>2</sup>, Vordermeier, H.M.<sup>3</sup>, Jemmi, T.<sup>1</sup>, Welsh, M.<sup>4</sup>, Keck, N.<sup>5</sup>, Whelan, A.<sup>3</sup>, Gormley, E.<sup>6</sup>, Boschioli, M.L.<sup>7</sup>, Moyen, J.L.<sup>8</sup>, Vela, C.<sup>9</sup>, Cagiola, M.<sup>10</sup>, Buddle, B.M.<sup>11</sup>, Palmer, M.<sup>2</sup>, Thacker, T.<sup>2</sup>, Oesch, B.<sup>12</sup>

<sup>1</sup> Federal Veterinary Office, Bern, Switzerland, <sup>2</sup> National Animal Disease Center, Agricultural Research Service, US Department of Agriculture, Iowa, USA,

<sup>3</sup> Veterinary Laboratory Agency, Addlestone, United Kingdom, <sup>4</sup> AFBI-Veterinary Sciences Division, Stormont, Northern Ireland, <sup>5</sup> Laboratoire Départemental Vétérinaire de l'Hérault, Montpellier, France, <sup>6</sup> UCD School of Veterinary Medicine, <sup>7</sup> Unité de Zoonoses Bactériennes, AFSSA-LERPAZ, Maisons-Alfort, France,

<sup>8</sup> Laboratoire Conseil Général de la Dordogne, France, <sup>9</sup> Ingenasa, Madrid, Spain, <sup>10</sup> Istituto Zooprofilattico dell'Umbria e delle Marche, Perugia, Italy,

<sup>11</sup> AgResearch, Palmerston North, New Zealand, <sup>12</sup> Prionics AG, Schlieren, Switzerland

### Veterinary Microbiology 151, 152-159 (2011)

Switzerland has been officially free of bovine tuberculosis (OTF) since 1960. Since 1980 the control of bovine tuberculosis (bTB) has been reduced to passive abattoir surveillance. Isolated cases of bTB, partly due to reactivation of human *Mycobacterium bovis* infections with subsequent transmission to cattle, have been noticed in the last years. In Europe, the overall prevalence of bTB is slightly increasing. Both OTF and non-OTF countries report increases in the proportion of bTB positive cattle herds. Current bTB eradication and control programs in Europe are facing a range of challenges. Whole herd depopulation is becoming a less attractive option for economic reasons and due to animal welfare concerns. Live animal trade is increasing both at national and international levels. Regarding these tendencies and taking into account the chronicity of bTB infection, pre-movement testing is becoming increasingly important as a central tool for eradication and for protection against re-introduction of bTB. Pre-movement testing, however specifically focuses on the infection status of individuals, requiring a high level of diagnostic accuracy to correctly diagnose infected animals. Current screening tests for bTB, however, have been designed to meet demands as herd tests. This illustrates that the modification of existing and/or the development of new diagnostics for bTB might be needed. The tuberculin skin test (TST), the primary screening test for bTB may in certain situations have low sensitivity. The interferon gamma (IFN- $\gamma$ ) assay is accepted to be more sensitive compared to TST. Reduced specificity, however, especially in areas of low bTB prevalence raises concerns. New antigen combinations including Rv3615c, OmpATb and others have been shown to complement ESAT-6 and CFP-10 in the whole blood IFN- $\gamma$  assay and resulted in improved sensitivity (compared to ESAT-6 and CFP-10) and specificity (compared to tuberculins). Lesion detection after slaughter represents a cost-effective procedure for passive surveillance of bTB, especially in areas of low prevalence or in regions free of bTB; however, its sensitivity is very low. This illustrates that trade is linked with a certain risk to re-introduce bTB in OTF regions or countries and that there may be delays in detecting a re-introduction of bTB. In conclusion, regarding the fact that some parameters linked with bTB programs are changing, the development of improved diagnostic tests with a high reliability for use as individual animal tests will be important for future eradication of bTB, in line with international commitment to high standard animal health programs.

*Reprinted from Veterinary Microbiology, 151, Schiller, I., Waters, W.R., Vordermeier, H.M., Jemmi, T., Welsh, M., Keck, N., Whelan, A., Gormley, E., Boschioli, M.L., Moyen, J.L., Vela, C., Cagiola, M., Buddle, B.M., Palmer, M., Thacker, T., Oesch, B., Bovine tuberculosis in Europe from the perspective of an officially tuberculosis free country: trade, surveillance and diagnostics, 152-159, Copyright 2011, with permission from Elsevier.*

## Quality control

### The relative effectiveness of, and reporting accuracy among, testers during field surveillance for bovine tuberculosis in Ireland

Clegg, T.A.<sup>1</sup>, Good, M.<sup>2</sup>, Duignan, A.<sup>2</sup>, More, S.J.<sup>1, 3</sup>

<sup>1</sup> UCD CVERA, <sup>2</sup> DAFM, <sup>3</sup> UCD School of Veterinary Medicine

Field surveillance using the Single Intradermal Comparative Tuberculin Test (SICTT) is potentially problematic, noting its reliance on a range of factors, including the skills and experience of the tester. The objective of this study is to quantify the relative effectiveness of, and reporting accuracy among, testers during field surveillance for tuberculosis in Ireland. All testers who carried out at least one annual herd test in 2006 are included in the study. Relative testing effectiveness will be assessed by comparing the number of observed and expected herd restrictions per tester. The latter will be predicted, using a logistic regression model and testers will be ranked, based on the ratio of observed and expected restrictions. Reporting accuracy will be based on the bovine measurements in SICTT negative animals. The study will also be extended to look at 2007 to 2010 data and to compare changes in the ranking of testers over time.

### A review of Irish tuberculin assays

Duignan, A.<sup>1</sup>, Costello E.<sup>2</sup>, Good M.<sup>1</sup>, Kenny K.<sup>2</sup>

<sup>1</sup> DAFM, <sup>2</sup> DAFM Veterinary Laboratory Service

Bovine tuberculin PPD has been described as a poorly defined, complex mixture containing more than 100 individual components in various stages of denaturation and is known to vary widely both in protein content and antigenic profile. The most reliable measure of the potency of a tuberculin is by assay in the species in which it will be routinely used. DAFM routinely conducts 2-3 potency assays on bovine tuberculin each year in cattle naturally infected with *M. bovis*. The trials are conducted by the DAFM Veterinary Laboratory Service on tuberculins chosen at random from the supply to be used in the field. The potency of a tuberculin is estimated by comparing the size of the reactions, elicited by an intradermal inoculation, to the size of the reactions of a 'standard' tuberculin of known potency. This project will give an outline of the evolution of PPD tuberculins and an account of assays carried out on Irish reactor cattle.

## Quality control in the national bovine tuberculosis eradication programme in Ireland

**Duignan, A.<sup>1</sup>, Good, M.<sup>1</sup>, More, S.J.<sup>2, 3</sup>**

<sup>1</sup> DAFM, <sup>2</sup> UCD CVERA, <sup>3</sup> UCD School of Veterinary Medicine

The Irish Bovine Tuberculosis (bTB) eradication programme operates under national legislation and fulfils the requirements of the EU Trade Directive 64/432. The programme includes annual single intradermal comparative tuberculin test (SICTT) screening of all herds, prompt removal of test reactors and further consequential retesting of herds. Continuous evaluation of all relevant activities is essential to deliver an effective national programme and to reassure all stakeholders that the highest possible standards are attained. Quality control (QC) is a recognised process in the delivery of quality products or services. This project will present a review of QC in the bTB eradication programme in Ireland, with particular emphasis on field surveillance. A broad range of programme elements subjected to QC, will be described, including personnel, training, equipment, tuberculins and laboratory. Particular attention will be paid to field surveillance (specifically, Private Veterinary Practitioner (PVP) performance).

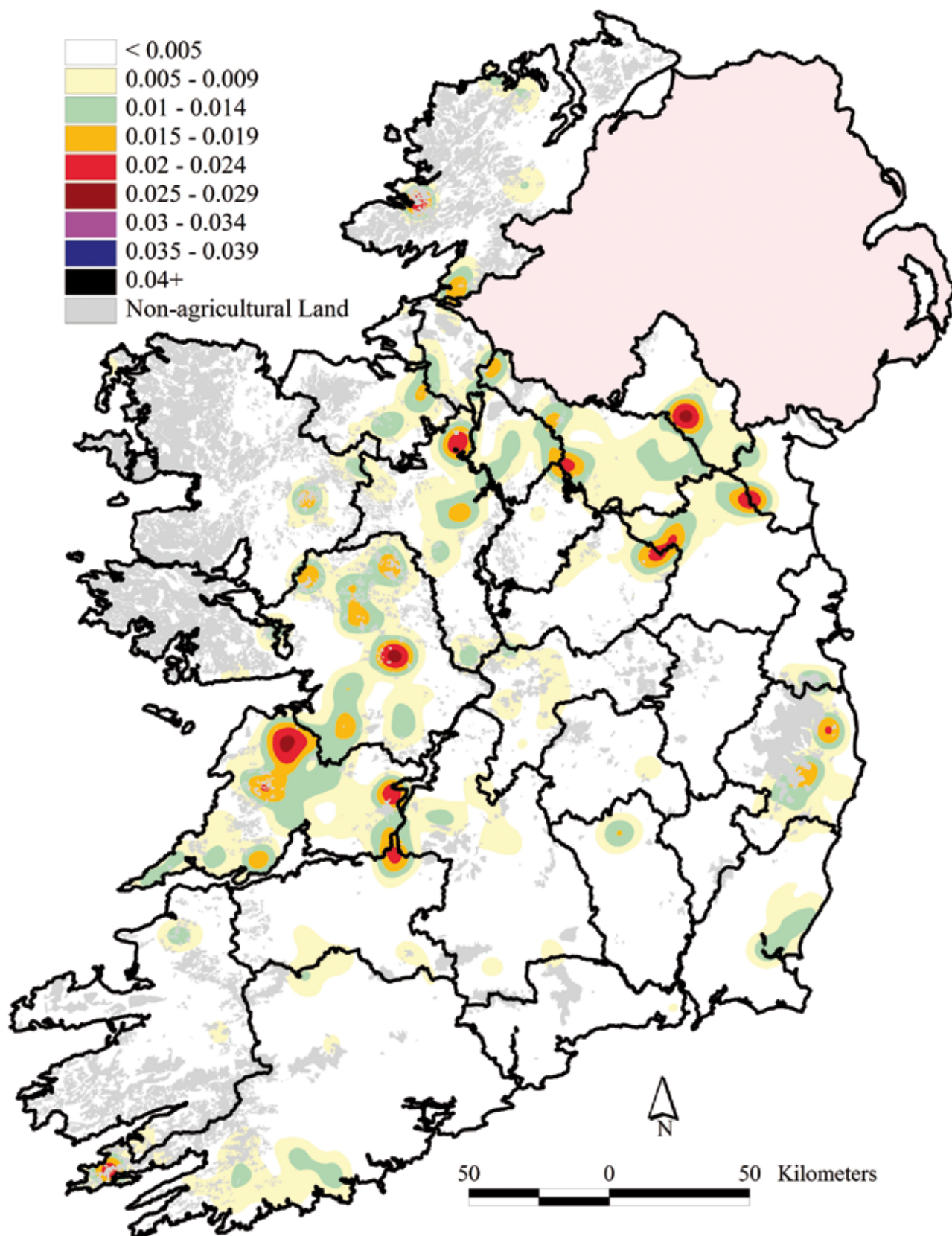
## Description of opinions among stakeholders of factors affecting the quality of SICTT testing in Ireland

**Meskell, P.<sup>1</sup>, Mulreany, M.<sup>2</sup>, More, S.J.<sup>3, 4</sup>**

<sup>1</sup> DAFM, <sup>2</sup> Institute of Public Administration, <sup>3</sup> UCD CVERA, <sup>4</sup> UCD School of Veterinary Medicine

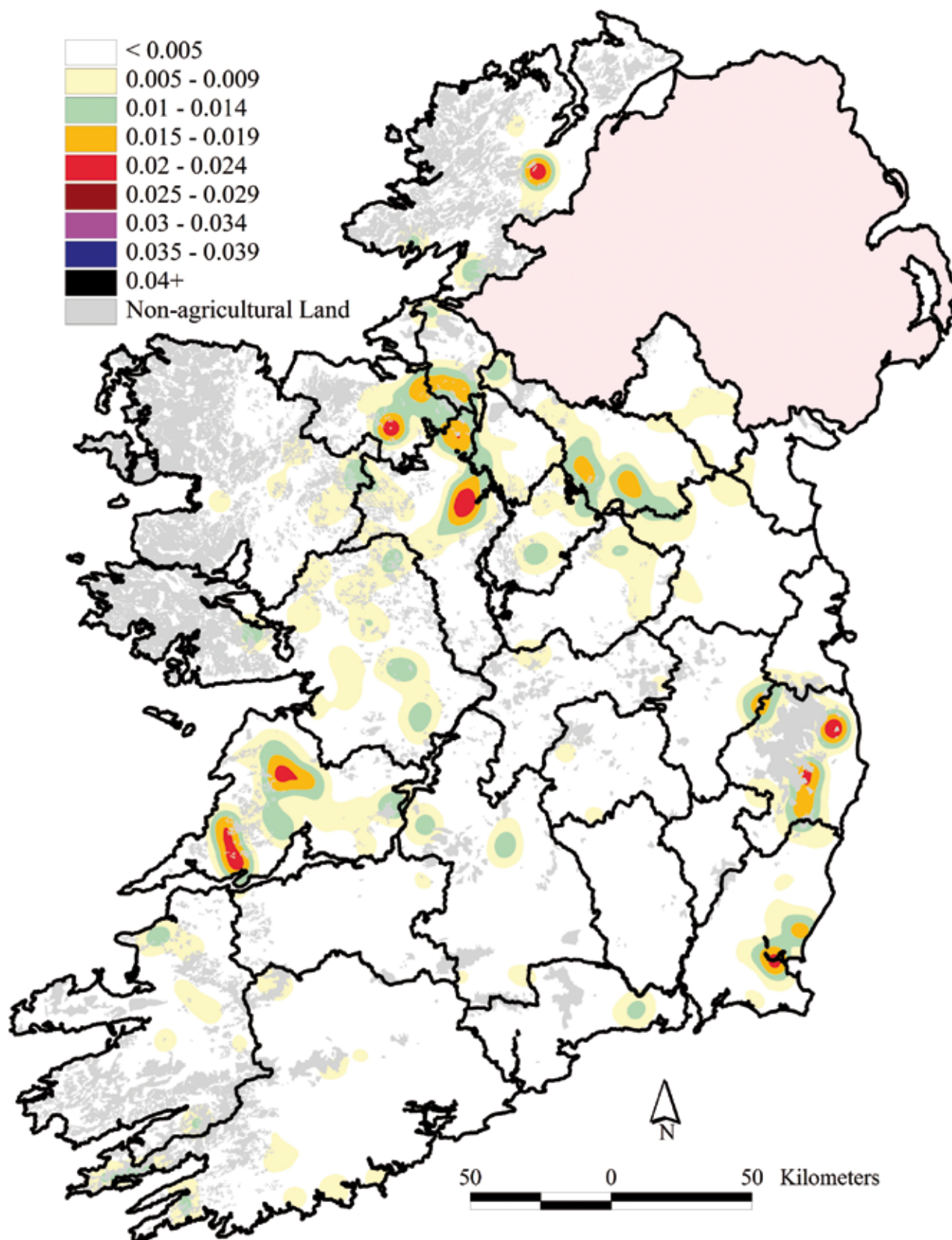
The single intradermal comparative tuberculin test (SICTT) is the primary method of field surveillance for bovine tuberculosis (bTB) in Ireland, and a number of methods are used to safeguard testing quality. As yet, little has been documented about the opinions of stakeholders on this issue. The purpose of this study is to describe opinions among stakeholders of factors affecting the quality of SICTT testing in Ireland. The study is being conducted using several qualitative methods, including focus groups and semi-structured interviews.

## Density of TB incidence



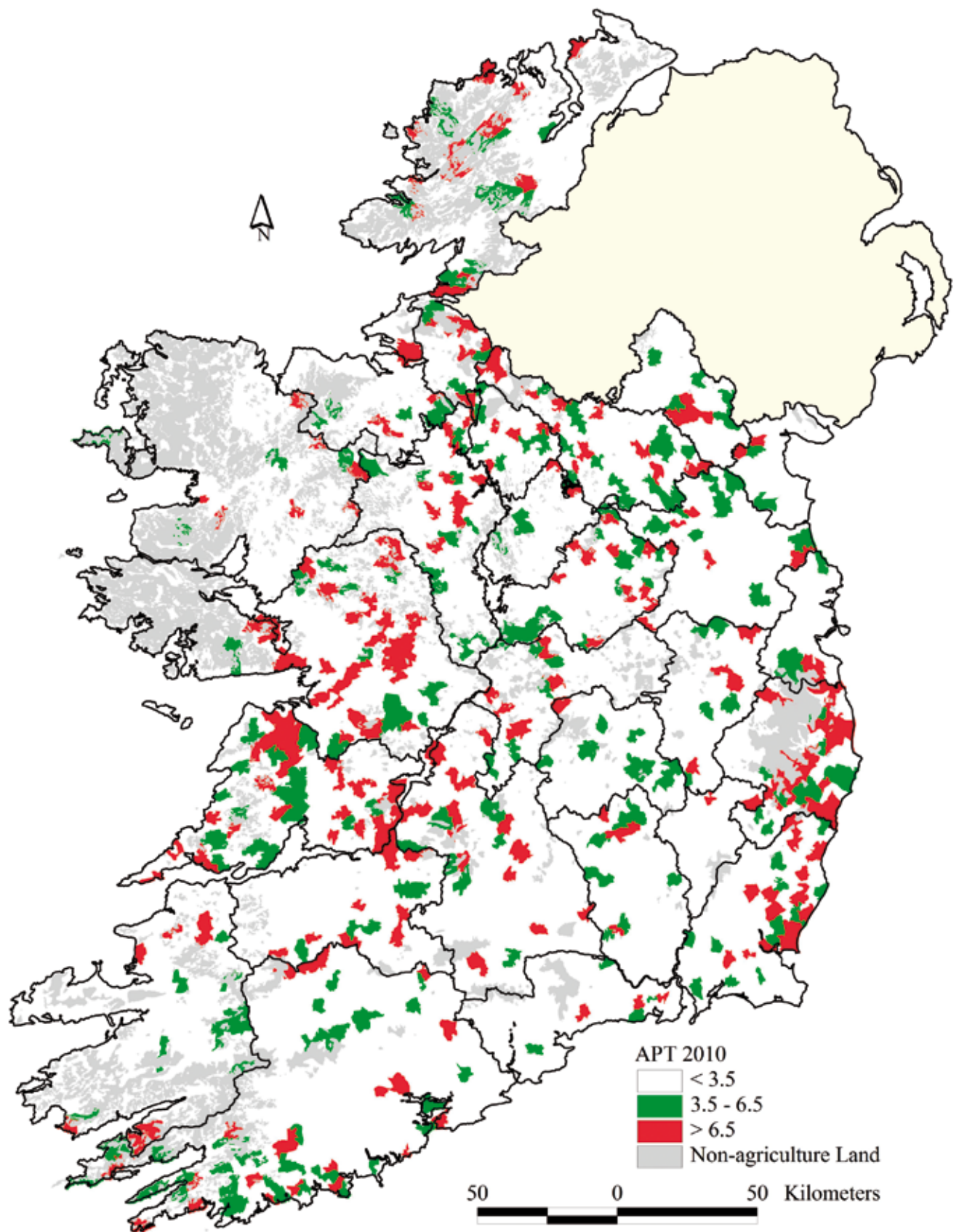
*Density of TB incidence per square km during 2010 (kernel density with search radius at 10km).*





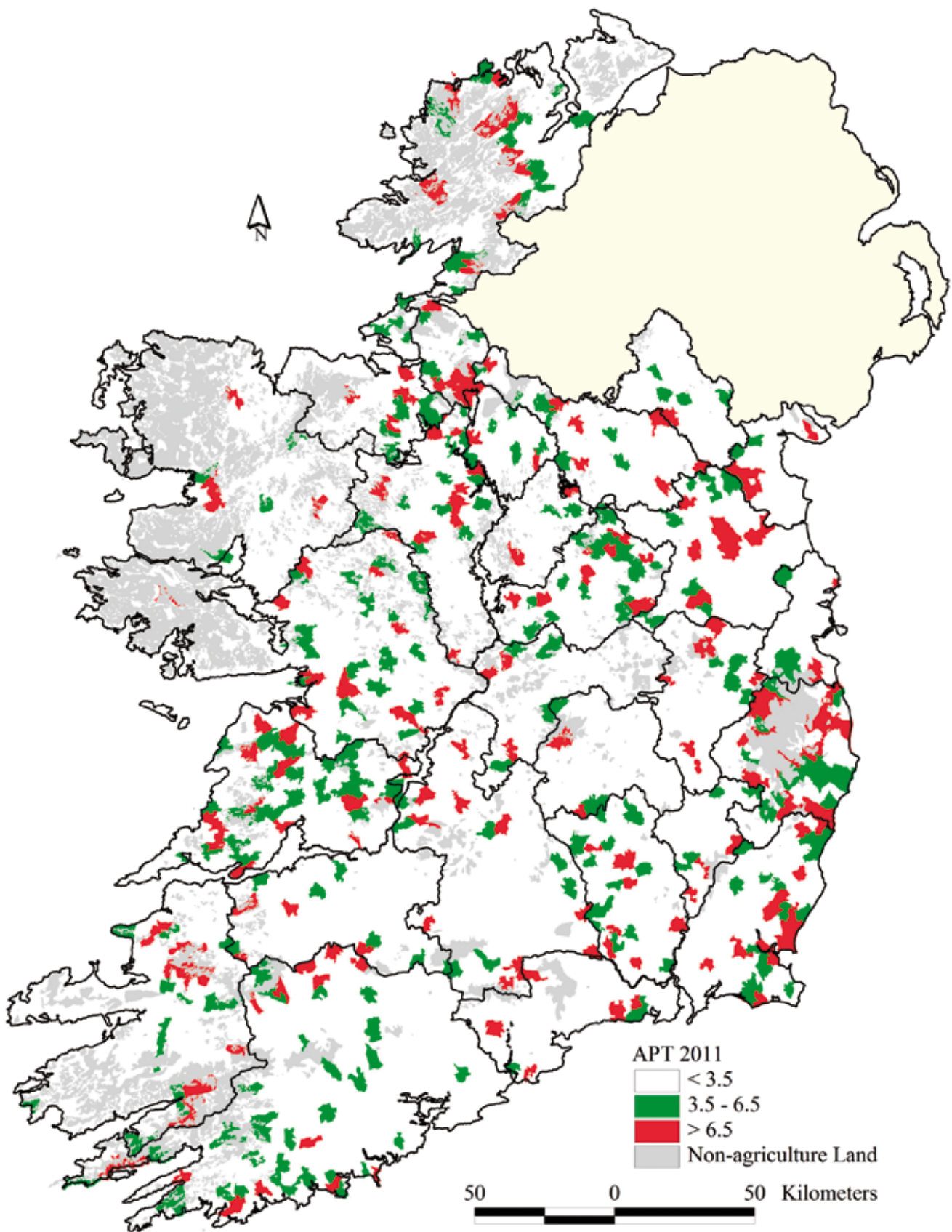
*Density of TB incidence per square km during 2011 (kernel density with search radius at 10km).*

## APT per DED



*APT (reactors per 1000 tests) per district electoral division, 2010.*



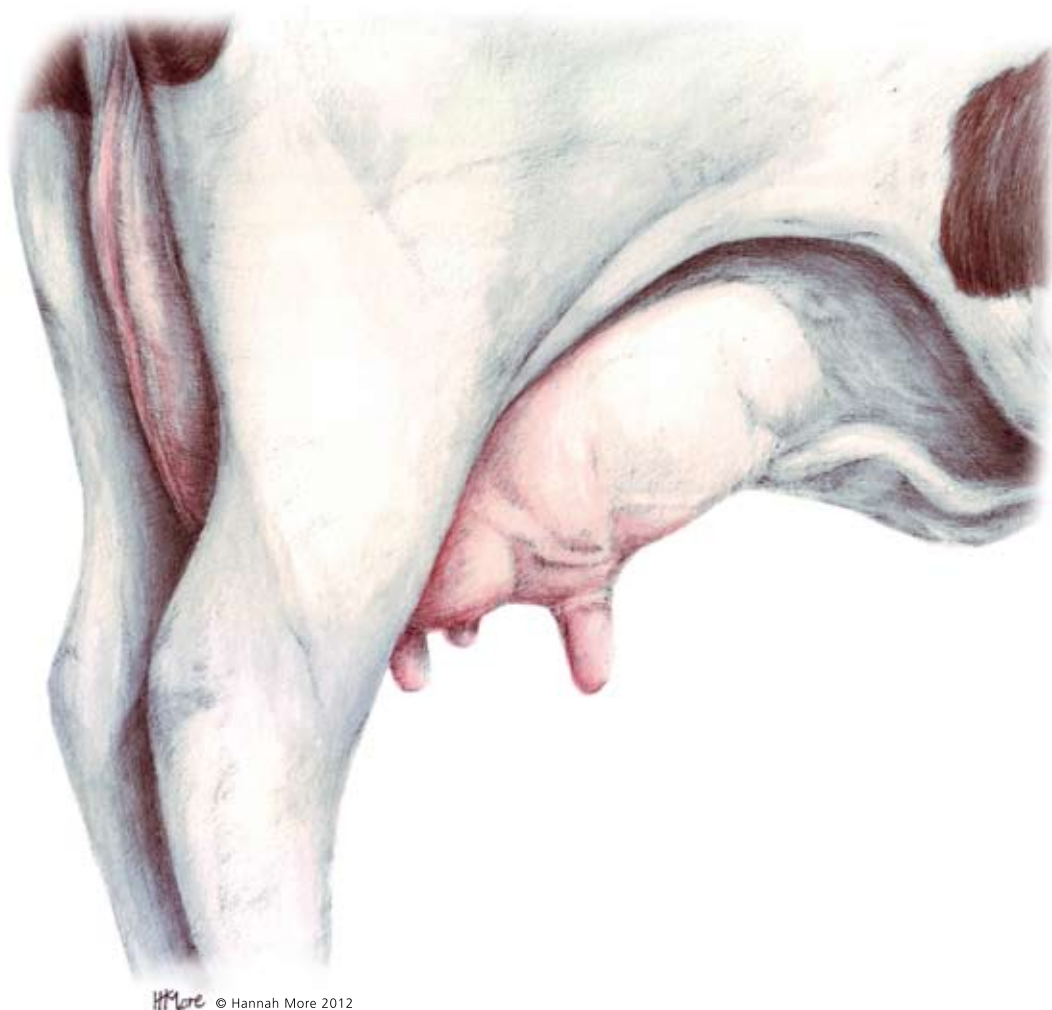


*APT (reactors per 1000 tests) per district electoral division, 2011.*









## Non-regulatory cattle health issues

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# Animal Health Ireland

## Food chain control - pre-harvest phase

More, S.J.<sup>1, 2</sup>

<sup>1</sup> UCD CVERA, <sup>2</sup> UCD School of Veterinary Medicine

A global approach is needed, from farm to table, to ensure that food for humans is safe to eat. The pre-harvest phase, during primary production, is the first step in the food chain. Controls at this stage of the food chain are critical. In this document, attention will be paid to three broad components of food chain control in the pre-harvest phase: the safety of animal feeds, animal health, and traceability. By necessity, this is a brief overview of a complex subject, and more-detailed reviews are available (including Smulders and Collins 2002).

## Animal Health Ireland: providing national leadership and coordination of non-regulatory animal health issues in Ireland

More, S.J.<sup>1, 2</sup>, Doherty, M.L.<sup>2</sup>, Downey, L.<sup>3</sup>, McKenzie, K.<sup>4</sup>, Devitt, C.<sup>3</sup>, O'Flaherty, J.<sup>5</sup>

<sup>1</sup> UCD CVERA, <sup>2</sup> UCD School of Veterinary Medicine, <sup>3</sup> Private consultant, <sup>4</sup> UCD School of Public Health, Physiotherapy and Population Science,

<sup>5</sup> Animal Health Ireland

**Revue scientifique et technique / Office international des épizooties (OIE Scientific and Technical Review) 30, 715-723 (2011)**

Livestock production plays an important role in the Irish economy. Regulatory animal health issues are the responsibility of government, but until recently there has been no national coordination of non-regulatory animal health issues. This gap has recently been filled with the establishment of Animal Health Ireland (AHI), a not-for-profit, partnership-based organisation providing national leadership and coordination of non-regulatory animal health issues in Ireland. Animal Health Ireland provides benefits to livestock producers and processors by providing the knowledge, education and coordination required to establish effective control strategies, both on-farm and nationally. This paper presents a brief overview of the context for AHI, and of its establishment and initial activities. Non-regulatory animal health issues have been prioritised. A series of work programmes (each focusing on a high-priority issue) have been established. Partnership is critical to success, both for AHI as an organisation and for effective farm-level transfer of knowledge. This model for national leadership and coordination of non-regulatory animal health issues may be of relevance elsewhere.

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## Setting priorities for non-regulatory animal health in Ireland: results from an expert Policy Delphi study and a farmer priority identification survey

More, S.J.<sup>1,2</sup>, McKenzie, K.<sup>3</sup>, O'Flaherty, J.<sup>4</sup>, Doherty, M.L.<sup>2</sup>, Cromie, A.R.<sup>5</sup>, Magan, M.J.<sup>4</sup>

<sup>1</sup> UCD CVERA, <sup>2</sup> UCD School of Veterinary Medicine, <sup>3</sup> UCD Institute for Food and Health, <sup>4</sup> Animal Health Ireland, <sup>5</sup> Irish Cattle Breeding Federation

### Preventive Veterinary Medicine 95, 198-207 (2010)

Agriculture is a very important contributor to the Irish economy. In Ireland, national animal health services have been a government, rather than an industry, responsibility. In 2009, Animal Health Ireland (AHI) was established to provide a partnership approach to national leadership of non-regulatory animal health issues (those not subject to national and/or EU regulation). The objectives of this study were to elicit opinion from experts and farmers about non-regulatory animal health issues facing Irish livestock industries, including prioritisation of animal health issues and identification of opportunities to maximise the effective use of AHI resources. The study was conducted with experts using Policy Delphi methodology over three rounds, and with farmers using a priority identification survey. Non-regulatory bovine diseases/conditions were prioritised by both experts and farmers based on impact and international competitiveness. For each high-priority disease/condition, experts were asked to provide an assessment based on cost, impact, international perception, impediment to international market access and current resource usage effectiveness. Further information was also sought from experts about resource allocation preferences, methods to improve education and coordination, and innovative measures to improve prevention and management. There was close agreement between responses from experts and dairy farmers: each gave highest priority to 3 diseases with a biosecurity risk (subsequently termed 'biosecure diseases') (bovine viral diarrhoea [BVD], infectious bovine rhinotracheitis [IBR], paratuberculosis) and 4 diseases/conditions generally without a biosecurity risk ('non-biosecure diseases/conditions') (fertility, udder health/milk quality, lameness, calf health). Beef farmers also prioritised parasitic conditions and weanling pneumonia. The adverse impact of biosecure diseases is currently considered relatively minor by experts, but would increase substantially in time. There are already substantial costs to farms and agribusiness from non-biosecure diseases/conditions. Experts preferred an equal allocation of resources between these biosecure and non-biosecure diseases/conditions, with emphasis on adopting/adapting international models, education and awareness-raising. The results from this study provide robust insights about non-regulatory animal health priorities in Ireland, as perceived by experts and farmers, using methodologies that are both transparent and inclusive. They have already been extremely influential in shaping national policy, as a foundation for interdisciplinary (and multi-agency) cooperation, as a contribution to efforts to encourage stakeholder responsibility-taking, and to ongoing development of postgraduate and undergraduate veterinary education in Ireland.

*Reprinted from Preventive Veterinary Medicine, 95, More, S.J., McKenzie, K., O'Flaherty, J., Doherty, M.L., Cromie, A.R., Magan, M.J., Setting priorities for non-regulatory animal health in Ireland: results from an expert Policy Delphi study and a farmer priority identification survey, 198-207, Copyright 2010, with permission from Elsevier.*

## Biosecure diseases

### *Biosecurity*

#### Design of a model for prediction of the probability of introduction of infectious agents into animal populations

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The consequences of disease introduction into animal populations are increasingly understood. Quantitative risk assessment models for disease introduction have been described for national import risk analysis but their use at individual farm level is limited. We describe the design of a quantitative deterministic model to estimate the probability of introduction of infectious disease into susceptible individual farm populations. The model needs to take account of three components: (1) exposure of the farm animals to external, potential sources of infection, (2) the presence of infectious agent in the potential infection source, and (3) exposure sufficient to support transmission to a farm animal. The first of these components is influenced by farm-specific variables, the third by agent-specific variables and the second by both. This model offers advantages over existing risk assessment tools. Total probability of disease introduction and probability of introduction from a single farm variable can be calculated and used for benchmarking herds and to identify high risk farm variables respectively.

#### Bioexclusion of diseases from dairy and beef farms: Risks of introducing infectious agents and risk reduction strategies

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Infectious diseases represent a major threat to the health and welfare of cattle herds internationally. Introduction of infectious agents into dairy and beef farms may be either through direct (purchased cattle, movement of resident cattle and contact with contiguous cattle) or indirect (visitors, fomites, biological materials and the environment) transmission routes. In this review, the evidence supporting these transmission routes for the introduction of infectious diseases is presented. In the absence of eradication programmes for many endemic infectious diseases, bioexclusion is the key management process to reduce the risks that they present. Various ameliorative bioexclusion strategies have been recommended in the literature and the evidence supporting these protocols is also reviewed here.



## Johne's disease

### Risk factors associated with Johne's disease test status in dairy herds in Ireland

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**Veterinary Record 168, 410 (2011)**

Johne's disease is a chronic, granulomatous enteritis of ruminants caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP). Although the recent prevalence and economic impact of clinical and subclinical Johne's disease in Irish dairy herds have been reported, there are no published studies examining the risk factors for detection of MAP by individual faecal culture (IFC) in Irish dairy herds. This short communication describes a case-control study aiming to identify the risk factors associated with detection of MAP by IFC in Irish dairy herds. Case herds ( $n=86$ ) were defined as herds with one or more IFC-MAP-positive results in the DAFM Veterinary Laboratory Service database from samples voluntarily submitted by private veterinary practitioners between 1995 and 2007. Control herds ( $n=125$ ) were defined as herds with no IFC-MAP-positive results in the DAFM Veterinary Laboratory Service database in the same period and with no ELISA-positive animals (over 12 months of age) in whole-herd sampling carried out as part of a national serosurvey in 2005. A questionnaire was designed to collect data on herd and management factors plausibly linked to the detection of MAP in dairy herds. The questions related to practices carried out before and after the year of MAP detection in the case herds and the same year (the reference year) in the control herds. Proportionally, more case herd owners operated a dairy-only enterprise compared with control herd owners. There was no geographical clustering in the distribution of case and control herds. Three significant risk factors (cattle importation, depopulation and herd size) and one protective factor (borrowing colostrum) remained in the final model. With the impending abolition of milk quotas in the European Union in 2015, many dairy herds are likely to be expanded. This study highlights the potential biosecurity risks for Johne's disease associated with herd expansion.

*Adapted from Veterinary Record, Barrett, D.J., Mee, J.F., Mullowney, P., Good, M., McGrath, G., Clegg, T., More, S.J., 168, 410, Copyright 2011, with permission from BMJ Publishing Group Ltd.*

### Genetic associations between Johne's disease and susceptibility to *Mycobacterium bovis* and *Mycobacterium avium* subsp *avium* in Irish Holstein Friesian dairy cows

Bermingham, M.L.<sup>1</sup>, More, S.J.<sup>2, 3</sup>, Good, M.<sup>4</sup>, Cromie, A.R.<sup>5</sup>, Mullowney, P.<sup>4</sup>, Higgins, I.M.<sup>2</sup>, Berry, D.P.<sup>6</sup>

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Johne's disease in cattle is caused by *Mycobacterium avium* subsp *paratuberculosis* (MAP). A recent study demonstrated that significant genetic variation exists for susceptibility to MAP infection in Irish Holstein Friesian dairy cows. Nevertheless, data on Johne's disease occurrence is not collected routinely on Irish dairy farms. The objective of this study was to estimate the genetic associations between resistance to MAP infection and measures of susceptibility to *M. bovis* and *M. avium* subsp *avium* infection. Serological response to MAP was used as a measure of cow susceptibility to Johne's disease. The single intradermal comparative tuberculin test was used as a measure of susceptibility of cows to *M. bovis* and *M. avium* infection. A total of 4,581 cow serological response to MAP records, 19,663 *M. bovis*-PPD responsiveness records and 15,824 *M. avium*-PPD responsiveness records were available for inclusion in the analysis. Genetic and residual (co)variance components between serological response to MAP and susceptibility to *M. bovis*-PPD and *M. avium*-PPD responsiveness were estimated using bivariate linear animal models. Serological response to MAP was strongly positively genetically correlated ( $0.84 \pm 0.20$ ) with susceptibility to *M. avium*-PPD responsiveness.

Susceptibility to *M. avium*-PPD responsiveness was not genetically correlated ( $0.03 \pm 0.32$ ) with serological response to MAP. The results from study suggest that selection for reduced *M. avium*-PPD responsiveness may indirectly increase resistance to MAP infection within the national Holstein Friesian dairy herd.

## Genetic variation in serological response to *Mycobacterium avium* subspecies *paratuberculosis* and its association with performance in Irish Holstein-Friesian dairy cows

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**Livestock Science 131, 102-107 (2010)**

Paratuberculosis, also referred to as Johne's disease, is a contagious and chronic disease in ruminants caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP). Few estimates of the genetic variation in measures of susceptibility to MAP are available in the literature and even less have attempted to elucidate the genetic associations between measures of susceptibility to MAP and performance in dairy cattle. The objectives of this study were to estimate the genetic variation in serological response to MAP in 4,789 Holstein-Friesian dairy cows from 44 Irish dairy herds, and to quantify its genetic association with performance traits measured in the first three lactations of genetically related animals. Univariate mixed linear and threshold animal models were used to estimate variance components and genetic correlations were estimated using bivariate animal linear mixed models; MAP serological response was treated as a continuous variable and dichotomous variable. The prevalence of MAP in the sample population was 4.4%. This figure cannot be extrapolated to the national dairy herd as the sample population was biased towards herds with increased likelihood of MAP infection. Estimates of heritability for MAP serological response varied from 0.07 to 0.15 depending on the model of analysis and whether serological response was treated as continuous or binary; standard errors varied from 0.024 to 0.062. Genetic correlations between MAP serological response and lactation milk, fat and protein yield were negative or close to zero although not always more than two standard errors from zero; stronger negative genetic correlations were evident in older parity animals. Serological response to MAP was not genetically correlated with milk fat concentration but was positively genetically correlated with milk protein concentration in first lactation and negatively correlated with calving interval. There was little or no genetic association between serological response to MAP and survival. Results from this study corroborate previous international suggestions that selection for reduced serological response to MAP is possible, although this does not necessarily imply a concurrent selection for either reduced prevalence of clinical disease or increased resistance to MAP infection.

*Reprinted from Livestock Science, 131, Berry, D.P., Good, M., Mullowney, P., Cromie, A.R., More, S.J., Genetic variation in serological response to Mycobacterium avium subspecies paratuberculosis and its association with performance in Irish Holstein-Friesian dairy cows, 102-107, Copyright 2010, with permission from Elsevier.*

## Control of Johne's disease: an international review

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National bovine Johne's disease (JD) control programmes exist in several countries including Australia, Canada, Denmark, the Netherlands, the United Kingdom and the United States of America. Programme design varies with industry specific variables, the goals of the programme and the nature of the implementing body. Important differences also arise from the necessary subjective interpretation of incomplete scientific literature in areas including test reliability and epidemiology. Organisations developing new JD control programmes can benefit from the combined experiences of existing national control programmes. This article reviews the structure (laboratory and non-laboratory herd screening, herd classification, intervention guidelines, ability to respond to new scientific developments), implementation and limitations of existing JD control programmes to inform the development of a voluntary national programme in Ireland.

## Evaluation of alternative testing strategies for estimating likelihood of infection with Johne's disease in Irish cattle herds

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Animal Health Ireland (AHI) is facilitating national discussion and action on a range of non-regulatory animal health issues in Ireland, including Johne's disease (JD). A technical working group is currently working to develop the framework for a voluntary national JD control programme, drawing on international and national science and best-practice. The programme will involve herd classification. However, testing requirements for initial herd screening and subsequent testing for JD in suckler and dairy herds in Ireland are currently not known. Some data are available from earlier scientific work (Sergeant *et al.* 2008; Tavoranpanich *et al.* 2008) and country programmes, however, these are not directly applicable to Ireland. The objective of the current project is to develop and test an epidemiological model to evaluate a range of testing strategies in an Irish context, with a focus on detection probability (given a specified design prevalence) and cost effectiveness. A simulation model was developed in the programming language R. Key model inputs included test sensitivity and specificity estimates, the design prevalence, testing options and testing costs. Only three tests are considered in the model (the individual serum ELISA, the individual milk ELISA and the faecal culture), as these are the only tests where sufficiently robust scientific data are available through international peer reviewed publication. Key model outputs include SeH (the probability that infection will be detected, if present at the design prevalence or greater) and ProbF (the probability that infection in the herd is either absent or at very low prevalence (less than the design prevalence)). ProbF, which is influenced by SeH, the prior probability of infection and the probability of introduction, could form the basis for herd classification.

## Bovine viral diarrhoea (BVD)

### Considerations on BVD eradication for the Irish livestock industry

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**Irish Veterinary Journal 64, 12 (2011)**

Animal Health Ireland has produced clear guidelines for the control of Bovine Viral Diarrhoea (BVD) infection in Irish cattle herds. In the course of developing these guidelines it was clear that a framework for regional and/or national BVD control would be required to increase the uptake of BVD control at farm level and reduce the overall prevalence of the disease. This paper assessed the economic impact of BVD, epidemiological aspects of the disease to its control, models of BVD control and international experiences of BVD control programmes. The technical knowledge and test technology exists to eradicate BVD. Indeed, many countries have done so successfully and others are embarking on control of the disease. The identification and prompt elimination of PI cattle will form the basis of any control programme. The trade of such animals must be curtailed. Pregnant and potentially pregnant bovines carrying PI fetuses pose a significant threat. International experience indicates systematic, well coordinated programmes have the most success, while voluntary programmes can make good initial progress but ultimately fail. The farming community must buy into any proposed programme, and without their support, failure is likely. To buy into the programme and create such a demand for BVD control, farmers must first be well informed. It is likely that stemming economic loss and improving productivity will be the primary motivator at individual farm level.

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### Aspects of bovine viral diarrhoea virus seroprevalence and vaccination in dairy and beef herds in the Republic of Ireland

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Bovine-viral diarrhoea (BVD) is an infectious disease of cattle with a worldwide distribution. Herd-level prevalence varies among European Union (EU) member states, and prevalence information facilitates decision-making and progress-monitoring with regard to control and eradication programmes. The primary objective of the present study was to describe aspects of herd BVD seroprevalence (based on pooled sera) and control on Irish farms, including vaccine usage. Preliminary validation of an indirect BVD antibody ELISA test (SVANOVA, Biotech AB, Uppsala, Sweden) using pooled sera was conducted as part of the present study. This test was then used in a cross-sectional study of a stratified random sample of 1171 Irish dairy and beef cow herds in 2009, for which vaccination status was determined by telephone survey, and the seroprevalence of BVD in Ireland was estimated in non-vaccinating herds. Comparison of herd-level classification was conducted in a subset of 111 dairy herds using the same ELISA on bulk milk tank (BMT) samples. Associations between possible risk factors (herd size (quartiles)) and herd-level prevalence were determined using chi-squared analysis. The herd-level BVD prevalence in non-vaccinating herds was 98.7% (95% CI - 98.3-99.5%) in the cross-sectional study with no significant difference between dairy and beef herds (98.3% vs 98.8%, respectively,  $p=0.595$ ). 95.4% agreement in herd classification of seroprevalence was found with bulk milk compared to serum pool results in non-vaccinating herds. 19.2 percent of farmers used BVDV vaccine; 81% of vaccinated herds were dairy. The results from this study indicate that the true herd-level seroprevalence to Bovine Virus Diarrhoea (BVD) virus in Ireland

is approaching 100%. The present study will assist Animal Health Ireland (AHI), an industry-government partnership charged with the national leadership and coordination of non-regulatory infectious diseases in Ireland, by providing useful information to guide policy and evaluate progress towards control.

## Cost benefit analysis of Irish BVD eradication programme

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Bovine viral diarrhoea virus (BVDV) causes an economically important endemic disease (BVD) of cattle in Ireland and worldwide. Systematic eradication by detection and removal of infectious (BVDV carrier) cattle has been successful in several regions. We therefore assessed the benefits (disease losses avoided) and costs (testing and culling regime) of a possible future eradication programme in Ireland. Published bio-economic models of BVDV spread in beef suckler herds and dairy herds were adapted to estimate the benefits of eradication in Ireland. A simple model of BVDV spread in beef finisher herds was devised to estimate the benefits of eradication in this sector. A six year eradication programme consisting of 5 inter-related virological and serological testing programmes is outlined and costed. We found that the annualised benefits of BVDV eradication in Ireland exceeded the costs by a factor of 5 in the beef suckler sector and a factor of 14 in the dairy sector. Corresponding payback periods were 1.2 and 0.5 years respectively. These results highlight the significant economic impact of BVD virus on the Irish cattle industry and suggest a clear economic benefit to eradication using the proposed approach. This type of cost-benefit analysis is considered an essential prerequisite prior to undertaking an eradication campaign of this magnitude.

## *Infectious bovine rhinotracheitis (IBR)*

### Aspects of bovine herpesvirus-1 infection in dairy and beef herds in the Republic of Ireland

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**Acta Veterinaria Scandinavica 53, 40 (2011)**

Infection with bovine herpesvirus-1 (BHV-1) causes a wide range of disease manifestations, including respiratory disease and abortion, with world-wide distribution. The primary objective of the present study was to describe aspects of BHV-1 infection and control on Irish farms, including herd-level seroprevalence (based on pooled sera) and vaccine usage. The characteristics of a diagnostic indirect BHV-1 antibody ELISA test when used on serum pools were evaluated using laboratory replicates for use in the seroprevalence study. The output from this indirect ELISA was expressed as a percentage positivity (PP) value. A proposed cut off (PCO) PP was applied in a cross-sectional study of a stratified random sample of 1,175 Irish dairy and beef cattle herds in 2009, using serum pools, to estimate herd seroprevalence. The study was observational, based primarily on the analysis of existing samples, and only aggregated results were reported. For these reasons, ethical approval was not required. Bulk milk samples from a subset of 111 dairy herds were analysed using the same ELISA. Information regarding vaccine usage was determined in a telephone survey. A PCO PP of 7.88% was determined to give 97.1% sensitivity and 100% specificity relative to the use of the ELISA on individual sera giving maximization of the prevalence independent Youden's index, on receiver operating characteristics analysis of replicate results. The herd-level BHV-1 seroprevalence was 74.9% (95% CI - 69.9%-79.8%), with no significant difference between dairy and beef herds. 95.5% agreement in herd classification was found between bulk milk and serum pools. Only 1.8 percent

of farmers used BHV-1 marker vaccine, 80% of which was live while 75% of vaccinated herds were dairy. A significant association was found between herd size (quartiles) and seroprevalence (quartiles). The results from this study indicate BHV-1 infection is endemic, although BHV-1 vaccines are rarely used, in the cattle population in Ireland.

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## Dynamics of individual animal Bovine Herpes Virus-1 antibody status on 9 commercial dairy herds

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### Research in Veterinary Science (in press)

Bovine Herpes Virus 1 (BoHV-1) is an important viral disease of cattle worldwide. In endemically infected herds, there is an incomplete understanding of the epidemiology of BoHV-1 infection. We describe the dynamics of animal-level BoHV-1 antibody status on 9 endemically infected commercial dairy herds, based on the results of serial milk antibody testing. Results were used to identify primary exposure, secondary exposure (from re-activation or re-exposure) and development of test-negative latent carrier (TNLC) status. 4,153 test results from 828 cow-lactations were analysed. Primary exposure occurred on two herds, secondary exposure occurred on all herds and development of TNLC status occurred in eight herds. Incidence of secondary exposure reduced over time and may have been related to increasing time since parturition. Regular secondary exposure is required to maintain measurable antibody status.

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## Leptospirosis

### Seroprevalence of *Leptospira* Hardjo in the Irish suckler cattle population

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Prior to the present study, the seroprevalence of leptospirosis in Irish suckler herds was unknown. In this study, we describe the herd and animal-level prevalence of *Leptospira* Hardjo infection in the Irish suckler cattle population. For the purposes of the study, the 26 counties of the Republic of Ireland were divided into 6 regions from which a representative number of herds were selected. A herd was considered eligible for sampling if it was not vaccinating against leptospirosis and if it contained  $\geq 9$  breeding animals of beef breed  $\geq 12$  months of age. In total, 288 randomly selected herds were eligible for inclusion in the seroprevalence dataset analysis. Serological testing was carried out using a commercially available monoclonal antibody-capture ELISA, (sensitivity 100%; specificity 86.67%). Herds were categorised as either "free from infection" or "infected" using the epidemiological software tool, FreeCalc 2.0. Using this classification, 237 herds were "infected" (82.29%). The South West and South East regions had the highest herd prevalence. The regional effect on herd prevalence was largely mirrored by breeding herd size. A true animal-level prevalence of 41.75% was calculated using the epidemiological software tool, TruePrev. There was a statistically significant regional



trend, with true prevalence being highest in the South East ( $P<0.05$ ). The median Breeding Herd Size (BHS), when categorised into quartiles, had a statistically significant influence on individual animal true seroprevalence ( $P<0.001$ ); true seroprevalence increased with increasing BHS. Leptospirosis is a widespread endemic disease in the Republic of Ireland. It is possible that economic losses due to leptospirosis in unvaccinated Irish suckler herds may be underestimated.

## Herd-level risk factors associated with *Leptospira* Hardjo seroprevalence in the Republic of Ireland

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The aim of the present study was to investigate risk factors for herd seropositivity to *Leptospira* Hardjo in Irish suckler herds. Herds were considered eligible for the study if they were unvaccinated and contained  $\geq 9$  breeding animals of beef breed which were  $\geq 12$  months of age. The country was divided into six regions using county boundaries. Herd and individual animal prevalence data were available from the results of a concurrent seroprevalence study. Herds were classified as "free from Infection" or "infected" at a minimum expected 40% within-herd prevalence. Questionnaires were posted to 320 herds chosen randomly from 25 counties in the Republic of Ireland. The questionnaire was designed to obtain information about vaccination; reproductive disease; breeding herd details; the presence of recognised risk factors from previous studies; and husbandry on each farm. Data collected from 128 eligible herds ( $n=128$ ) were subjected to statistical analysis. Following the use of Pearson's Chi-Square Test, those variables associated with a herd being "infected" with a significance level of  $P<0.2$  were considered as candidates for multivariable logistic regression modelling. Breeding herd size was found to be a statistically significant risk factor after multivariable logistic regression. The odds of a herd being positive for leptospiral infection were 5.47 times higher ( $P=0.032$ ) in herds with 14 to 23 breeding animals compared with herds with  $\leq 13$  breeding animals, adjusting for region, and 7.08 times higher ( $P=0.033$ ) in herds with 32.6 to 142 breeding animals. Breeding herd size was identified as a significant risk factor for leptospiral infection in Irish suckler herds, which was similar to findings of previous studies of leptospirosis in dairy herds.

## Non-biosecure diseases and conditions

### Milk quality

#### A HACCP-based approach to mastitis control in dairy herds. Part 1: Development

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**Irish Veterinary Journal 64, 2 (2011)**

Hazard Analysis and Critical Control Points (HACCP) systems are a risk based preventive approach developed to increase levels of food safety assurance. This is part 1 of a pilot study on the development, implementation and evaluation of a HACCP-based approach for the control of good udder health in dairy cows. The paper describes the use of a novel approach based on a deconstruction of the infectious process in mastitis to identify Critical Control Points (CCPs) and develop a HACCP-based system to prevent and control mastitis in dairy herds. The approach involved the creation of an Infectious Process Flow Diagram, which was then cross-referenced to two production process flow diagrams of the milking process and cow management cycle. The HACCP plan developed, may be suitable for customisation and implementation on dairy farms. This is a logical, systematic approach to the development of a mastitis control programme that could be used as a template for the development of control programmes for other infectious diseases in the dairy herd.

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## A HACCP-based approach to mastitis control in dairy herds. Part 2: Implementation and evaluation

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**Irish Veterinary Journal 64, 7 (2011)**

Part 1 of the study described the development of a Hazard Analysis and Critical Control Point (HACCP) based programme and accompanying handbook for the control of mastitis. This paper describes the implementation and evaluation of customised HACCP-based programmes, which were developed from the handbook and assessed on six Irish dairy farms. Both quantitative and qualitative (action research) research methodologies were used to measure the success of implementation and efficacy of control of sub-clinical mastitis as measured by Somatic Cell Counts (SCC) and the degree of compliance by farmers in adopting and maintaining recommendations throughout the course of the study period. No overall differences in SCC before and during the implementation of the study were found when all six farms were considered together. Three of the six study farms experienced a significant decrease in herd milk recorded SCC during the implementation of the control programme. An essential part of the study was achieving initial agreement on recommendations as well as ongoing monitoring of compliance during the study. This pilot study shows that HACCP can be implemented on farms as a means of working towards the control of mastitis and that farmer attitude, and understanding of mastitis are crucial in terms of motivation irrespective of practical approaches used to manage mastitis.

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## An estimation of the effect of dilution due to milk yield on milk somatic cell count across cow parities in a grass-based system

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This study investigates the influence of parity on the association between SCC and milk yield. It is well recognised, based on published international work, that high SCCs are associated with a drop in milk yield. As yet, however, there is limited understanding of the relative importance of this effect across different parities. The results are important, both from the perspective of improved scientific knowledge, and also to provide key parameters for the Teagasc Moorepark economic modeling of mastitis impact.

## The relationship between herd size in Ireland and milk somatic cell counts: A retrospective cohort study

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Studies examining the relationship between herd size and elevated SCC have conflicting reports of the association between the two. The main objective of this study was to determine the relationship between herd size in Ireland and elevated individual cow geometric mean 24 h milk SCC above 200,000 cells/ml [MSCC200] and above 400,000 cells/

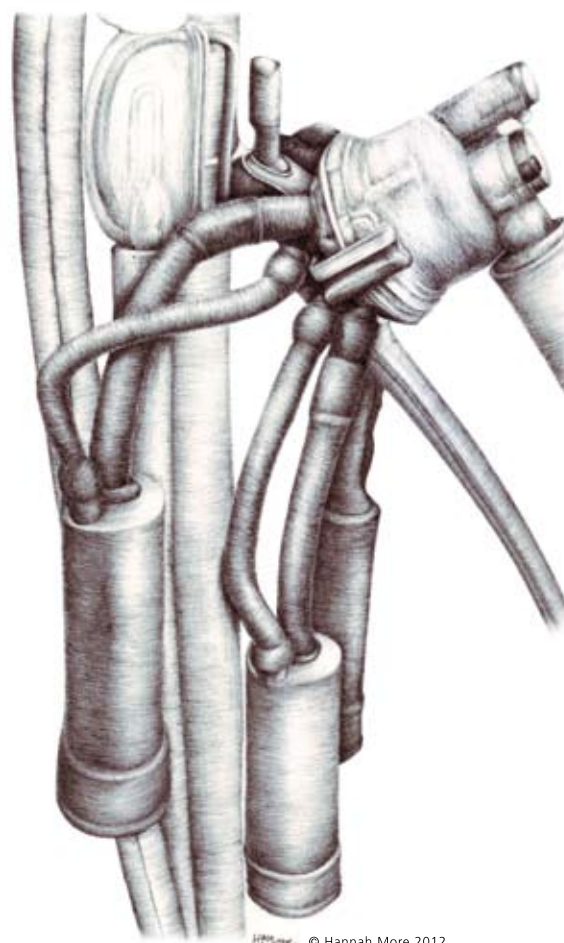
ml [MSCC400] in an entire lactation, and to determine the influence of herd expansion on these parameters. Individual cow monthly composite milk recordings from all Irish cows enrolled with ICBF were obtained for 2009. Multivariable random effect logistic regression models were fitted. The effect of herd expansion was examined by fitting an interaction term between herd size and herd expansion. Results show that 26.25% and 10.68% of cows had MSCC200 and MSCC400, respectively. The majority of these cows (62.59%) were from herds that had not expanded (33.45%) or had undergone  $\leq 15\%$  expansion (29.14%) in the past four years. Herd size was associated with MSCC200 and MSCC400 after adjusting for a number of variables, with the odds of MSCC200 and MSCC400 higher in larger herds. There was no interaction between herd size and herd expansion in the models fitted. In Ireland, larger milk recording herds may need to pay particular attention to their SCC status. Caution, however, should be taken when interpreting the results of the effect of herd size from this and other studies on SCC due to difference in determination and categorisation of herd size. Results also reveal that there was little herd expansion in these milk recording herds over the four years examined. However, this may change significantly in the near future given the growing global demand for dairy products.

## Drivers and constraints to improving milk quality in Ireland

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Approximately 85% of annual production from Ireland's dairy industry is exported, highlighting the need for ongoing improvement in quality in a competitive international trading environment. However, several recent studies have highlighted concerns with udder health/mastitis and associated intramammary antibiotic usage on Irish dairy farms. *CellCheck*, a national mastitis control programme, was launched by Animal Health Ireland (AHI) in late 2010. A process called 'terrain-mapping' was conducted to understand industry challenges and drivers to milk quality improvement. In-depth qualitative interviews were completed with  $n = 12$  representatives from different sectors of the Irish dairy industry. Disagreement exists in acknowledging individual stakeholder responsibility in contributing to milk quality improvement, and in agreeing on the best way forward. This is despite consensus on the need to redefine quality milk above current regulatory standards, and of the common recognition of opportunities. Disagreements translate into concerns about lack of commitment and active response to what should be an integrated industry-led response. Opportunities for incremental change in the Irish dairy sector are undermined by an emphasis on maintaining the private good over contributing to the public good. Animal Health Ireland provides an opportunity to build stakeholder agreement.



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## Current practices to manage milk quality on Irish dairy farms

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A pilot programme of farmer workshops was recently conducted as part of the national *CellCheck* milk quality programme. The objective of this workshop was to deliver best science & practice information around mastitis control to farmers, and to encourage the uptake of key best practices in everyday milking routines. An opportunity arose, as part of this pilot, to report current practices to manage milk quality on Irish dairy farms. This project reports these findings, with early results highlighting differences between the reported frequency of key behaviours, and the quality of these behaviours, including liner change and teat disinfection.

## Mapping milk production in Ireland

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This project seeks to present a national map of milk production based on data supplied by the Irish Cattle Breeding Federation for milk recording herds. A Geographical Information System will be employed to generate a triangular irregular network to create a surface that will be representative of the entire country based on a sample of approximately 6,000 herds.

## Insights into udder health and intramammary antibiotic usage on Irish dairy farms during 2003-2010

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This paper presents insights into udder health and intramammary antibiotic usage on Irish dairy farms during 2003-2010, based on data from several sources. Three data sources were used, including data on milk recording data, intramammary antibiotic sales and animal health assessment. The milk recording data included a single unadjusted herd-level somatic cell count (SCC) value for each herd at each milk recording, being the arithmetic mean of cow-level SCC of each cow at that recording, weighted by cow-level yield. These data were used to calculate the percentage of herds each month where the unadjusted herd SCC exceeded 200,000 and 400,000 cells/mL. Two logistic generalised estimating- equations (GEE) models were developed, the outcome variable being either the probability that the monthly SCC of a herd was greater than 400,000 cells/mL or less than or equal to 200,000 cells/mL. Spring herds had a lower probability of a high SCC (>400,000 cells/mL) during February to October compared to non-Spring herds but a higher probability between November to January. The odds of a high SCC were greater in 2005, 2006, 2009 and 2010 but less in 2007 and 2008 compared to 2004. Smaller herds had higher odds of having a high SCC compared to larger herds. We present the number of intramammary tubes and the quantity of active substance (kg) sold annually in Ireland during 2003-2010. We infer an incidence of clinical mastitis of 54.7 cases per 100 cow-years at risk, assuming 4 tubes per treatment regime, one affected quarter per cow, tubes restricted to clinical cases only and 100% of treated cases considered new cases, based on data collected on sales of in-lactation intra-mammary antibiotics. With differing assumptions, this estimate varied between 26.1 and 77.9 cases per 100 cow-years at risk. Using data on sales of dry cow therapy intra-mammary antibiotics, we also infer that most Irish dairy farmers use blanket dry cow therapy. Udder health is a concern on a number of Irish dairy farms. High SCC results were present throughout the year, but more marked towards the start and end of each milking season. Animal Health Ireland recently commenced a major national programme, *CellCheck*, in collaboration with a broad range of stakeholders, to support national SCC improvement.



## The effect of data adjustment for somatic cell counts, as outlined in EU legislation, on herd eligibility to supply raw milk for human consumption

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Regulatory SCC limits have been established in many dairy producing countries, to improve milk quality and herd productivity. Within the European Union, raw milk must comply with microbiological criteria and standards for plate count and somatic cell count, as required under EU Regulation 853/2004. In the current study, we examine the effect of data adjustment for somatic cell counts, as outlined in current EU legislation, on herd eligibility to supply raw milk for human consumption, using Irish data for illustration.

### Fertility

#### Breeding soundness evaluation of 36 bulls with reduced reproductive performance: a retrospective study

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The objective of this study was to determine the fertility status of 36 bulls presented with a history of reduced reproductive performance to University Veterinary Hospital, University College Dublin between 27/11/2006 and 14/03/2011. A full breeding soundness evaluation (BSE) was performed on all bulls by the same clinician at submission. Components of the BSE included a full general clinical exam, assessment of the locomotion system and assessment of the reproductive tract. Reproductive tract evaluation comprised measurement of the scrotal circumference (cm), assessment of testicles for shape, symmetry and consistency. The penis and prepuce were assessed by inspection and palpation and the accessory glands were assessed via rectal palpation. Subsequently a semen sample was obtained by electro-ejaculation. Following semen collection the parameters assessed were; volume of the ejaculate (ml), colour of the ejaculate (1=almost clear, 2=skim milk, 3=milk), sperm concentration ( $\times 10^6$ ), sperm motility (1 to 5), percentage live sperm (%) and percentage normal sperm (%). These results were used to classify animals as fertile, sub-fertile or infertile on the day of examination. A chi-square test was used to assess the unadjusted relationship between fertility classification and colour, and fertility classification and motility. Age at examination, scrotal circumference, semen volume, concentration, percentage live sperm and percentage normal sperm, all continuous variables were divided into categorical variables based on quartiles. The average age of bulls at examination was  $2.7 \pm 0.21$  years (range 1.2 – 5.8). None of the 36 bulls presented with physical abnormalities. After the BSE and semen evaluation, 15 were classified as fertile, 8 as sub-fertile and 13 as infertile. Colour ( $P < 0.001$ ) and motility ( $P = 0.05$ ) were significantly associated with fertility classification. After converting from continuous to categorical variables, concentration ( $P < 0.001$ ) and percentage live sperm ( $P = 0.002$ ) were also associated with fertility classification. Of those bulls classified as infertile, motile sperm were found in only 3 samples examined. In conclusion, BSE was performed on 36 bulls with questionable fertility. Of these 36 bulls, none were physically abnormal and 15/36 (42%) had normal semen. Of the parameters measured colour of the ejaculate, sperm motility as well as concentration and percentage live sperm were directly related to overall fertility outcome.

## Effect of body condition score at key reproductive stages and vaginal mucus score on production and reproduction parameters in dairy cows

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The objectives were two-fold: i) to investigate the effect of Body Condition Score (BCS) at calving and at 1st service on the following production and reproduction parameters: vaginal mucus score at 3-4 weeks post partum, calving-1st service, calving-conception, number of serves, peak daily milk yield, milk fat and protein at 1st milk recording, average milk yield during duration of the study, conception rates to 1st and 2nd service and overall pregnancy rate, and ii) to investigate the effect of vaginal mucus score at 3-4 weeks post partum on the same parameters, as well as total yield fat and protein. Ninety spring calving Friesian/Holstein cows on a commercial dairy farm were used. BCS was recorded on a scale from 1-5 at calving and at 1st service and vaginal mucus score was recorded between 3 and 4 weeks post partum on a scale from 0-3. All other production and reproduction parameters were collected at the end of the breeding season and all data were analysed using Chi square analysis. Cows with a BCS lower than 2.5 at calving tended to have a longer calving-1st service interval ( $p=0.068$ ). The group of cows that had a BCS of 2.5 had a significantly lower ( $p=0.04$ ) overall pregnancy rate and received more services than all other BCS groups. Cows with a BCS of 2.25 at 1st service had a lower pregnancy rate than the cows in all the other groups, but these cows also tended to have received less services per conception. Cows with a high vaginal mucus score at calving had a significant lower ( $p<0.05$ ) average daily milk yield in the experimental period and a significant lower total yield protein. In conclusion, low BCS had a negative effect on some of the reproductive parameters measured and uterine infection as measured by vaginal mucus score had a negative effect on some of the production parameters.

## Key factors affecting dairy cow fertility in Ireland - pilot study

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The analysis of herd management records allows for accurate assessment of the current status of the herd, a crucial decision making tool to implement effective change. The aim of this study is to evaluate the relative importance of some cow and management factors on reproductive indices in moderate yielding seasonal dairy herds. Breeding records of all cows were collected on 10 seasonal calving herds, during herd health visits, between 2007 and 2009. Individual cow identities ( $n = 1,174$ ), calving date, all service dates, lactation number, and pregnancy diagnosis outcomes were recorded for all milking cows. Logistic regression modelling will be used to determine the effect of cow factors including parity, timing of calving relative to start of breeding season and number of days post partum at first service, on reproductive indices. Herd factors including average heat detection efficiency and herd size will be investigated.

## Key factors affecting dairy cow fertility in Ireland - larger study

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The analysis of herd management records allows for accurate assessment of the current status of the herd, a crucial decision making tool to implement effective change. Monitoring of such changes to ensure their effectiveness is essential to the success of any programme, while, participation in discussion groups, allows for peer comparisons, a key factor in motivating herd management change. The aim of this paper is to calculate the fertility indices of herds recorded on

the Irish Cattle Breeding Federation (ICBF)'s database to enable effective evaluation of the fertility performance of the Irish dairy herd and to determine the key drivers of infertility in the Irish dairy herd. Breeding record data of the 2009 breeding season from all spring calving Irish dairy herds registered for ICBF's Herd Plus service, that had a minimum of six recordings during 2009, were collected from database. Individual cow identities (jumbo tag and national identifiers), date of birth, lactation number, Estimated Breeding Index (EBI; milk and fertility) 2009 calving date, all service dates, sire and inseminator for each service, pregnancy diagnosis outcomes, subsequent calving date, recorded milk yields, fat and protein concentrations at each recording period were gathered for all milking cows within each herd. Logistic regression modelling using the statistical package STATA will be utilized to analyse the dataset. Analyses will be conducted at animal level taking clustering within herd into account.

## Calf health

### Calf health from birth to weaning. I. General aspects of disease prevention

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**Irish Veterinary Journal 64, 10 (2011)**

Calfhood diseases have a major impact on the economic viability of cattle operations. This is the first in a three part review series on calf health from birth to weaning, focusing on preventive measures. The review considers both pre- and periparturient management factors influencing calf health, colostrum management in beef and dairy calves and further nutrition and weaning in dairy calves.

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### Calf health from birth to weaning. II. Management of diarrhoea in pre-weaned calves

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**Irish Veterinary Journal 64, 9 (2011)**

Calfhood diseases have a major impact on the economic viability of cattle operations. The second of this three part review series considers the management of diarrhoeic diseases in pre-weaned calves. In neonatal calf diarrhoea, oral rehydration therapy is the single most important therapeutic measure to be carried out by the farmer and is usually successful if instigated immediately after diarrhoea has developed. Continued feeding of milk or milk replacer to diarrhoeic calves is important, to prevent malnourishment and weight loss in affected calves. Indiscriminate antibiotic treatment of uncomplicated diarrhoea is discouraged, whereas systemically ill calves can benefit from systemic antibiotic treatment for the prevention of septicaemia or concurrent diseases. Ancillary treatments and specific preventive measures are discussed. Eimeriosis has a high economic impact on the farming industries due to direct cost of treatment and calf losses, but especially due to decreased performance of clinically as well as sub-clinically

affected animals. Emphasis lies on prophylactic or metaphylactic treatment, since the degree of damage to the intestinal mucosa once diarrhoea has developed, makes therapeutic intervention unrewarding.

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## Calf health from birth to weaning.

### III. Housing and management of calf pneumonia

Lorenz, I.<sup>1</sup>, Earley, B.<sup>2</sup>, Gilmore, J.<sup>3</sup>, Hogan, I.<sup>4</sup>, Kennedy, E.<sup>5</sup>, More, S.J.<sup>1,6</sup>

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**Irish Veterinary Journal 64, 12 (2011)**

Calfhood diseases have a major impact on the economic viability of cattle operations. A three part review series has been developed focusing on calf health from birth to weaning. In this paper, the last of the three part series, we review disease prevention and management with particular reference to pneumonia, focusing primarily on the pre-weaned calf. Pneumonia in recently weaned suckler calves is also considered, where the key risk factors are related to the time of weaning. Weaning of the suckler calf is often combined with additional stressors including a change in nutrition, environmental change, transport and painful husbandry procedures (castration, dehorning). The reduction of the cumulative effects of these multiple stressors around the time of weaning together with vaccination programmes (preconditioning) can reduce subsequent morbidity and mortality in the feedlot. In most studies, calves housed individually and calves housed outdoors with shelter, are associated with decreased risk of disease. Even though it poses greater management challenges, successful group housing of calves is possible. Special emphasis should be given to equal age groups and to keeping groups stable once they are formed. The management of pneumonia in calves is reliant on a sound understanding of aetiology, relevant risk factors and of effective approaches to diagnosis and treatment. Early signs of pneumonia include increased respiratory rate and fever, followed by depression. The single most important factor determining the success of therapy in calves with pneumonia is early onset of treatment, and subsequent adequate duration of treatment. The efficacy and economical viability of vaccination against respiratory disease in calves remains unclear.

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## Calf mortality in Ireland

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The objective of this study is to determine the mortality rate of all calves in Ireland born between 1st July 2009 and 31st June 2010, and to identify risk factors associated with calf mortality.









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## Other animal health and welfare issues

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## Animal welfare

### Bovine welfare

#### A review of bovine cases consigned under veterinary certification to emergency and casualty slaughter in Ireland during 2006 to 2008

Cullinane, M.<sup>1</sup>, O'Sullivan, E.<sup>2</sup>, Collins, G.<sup>1</sup>, Collins, D.M.<sup>3</sup>, More, S.J.<sup>3,4</sup>

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**Irish Veterinary Journal 63, 568-577 (2010)**

The emergency and casualty slaughter of cattle for human consumption (in cases where animals are likely to have suffered from acute or chronic pain, respectively) in Ireland requires that the animal is accompanied to the slaughterhouse by an official veterinary certificate (VC) completed on-farm by the owner's private veterinary practitioner (PVP). No published data is currently available in Ireland based on information provided in these VCs. In this paper, we present a review of bovine cases consigned under veterinary certification to emergency and casualty slaughter in Ireland during 2006 to 2008. All VCs during the years 2006 (where available), 2007 and 2008 were collected from four large Irish slaughterhouses. The data were computerized, and analysed using descriptive and spatial methods. In total, 1,255 VCs were enrolled into the study (1,255 study animals, 1,072 study herds), 798 (63.6%) and 457 (36.4%) animals were consigned to emergency and casualty slaughter, respectively. VCs were completed throughout the year, with consigned animals travelling a mean distance of 27.2 km from farm to slaughter. The time elapsed between veterinary certification and slaughter was greater than three days for 18.2% of all study animals. In 965 (76.9%) animals, the certified suspected disability related to the locomotory system, most commonly as a result of fractures. Among animals for which data were available, 11.9% were totally condemned at post-mortem. The transport of animals with fractured limbs and/or other painful conditions is a significant animal welfare concern.

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#### Veterinary certificates for emergency or casualty slaughter bovine animals in Ireland: are the welfare needs of certified animals adequately protected?

Cullinane, M.<sup>1</sup>, O'Sullivan, E.<sup>2</sup>, Collins, G.<sup>1</sup>, Collins, D.M.<sup>3</sup>, More, S.J.<sup>3,4</sup>

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All emergency and casualty bovines in the Republic of Ireland that are deemed to be fit for human consumption must be accompanied to the slaughterhouse by an official veterinary certificate (VC). In a previous study, Cullinane *et al.* (2010) conducted a review of bovine cases consigned under veterinary certification to emergency and casualty slaughter in Ireland during 2006 to 2008. The current study will further evaluate the original results, with emphasis on the period of validity, transport distance and transport conditions. It will also consider whether or not the current VC adequately protects the welfare needs of the certified bovine animal.

## Identification of key performance indicators for on-farm animal welfare incidents: possible tools for early warning and prevention

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The objective of this study was to describe aspects of case study herds investigated by the Department of Agriculture, Food and the Marine (DAFM) in which animal welfare incidents occurred and to identify key performance indicators (KPIs) that can be monitored to enhance the Early Warning System (EWS). Despite an EWS being in place for a number of years, animal welfare incidents continue to occur. Questionnaires regarding welfare incidents were sent to Superintending Veterinary Inspectors (SVIs), resulting in 18 herds being chosen as case study herds, 12 of which had a clearly defined welfare incident date. For each study herd, data on six potential KPIs were extracted from DAFM databases. The KPIs for those herds with a clearly defined welfare incident date were studied for a consecutive four year window, with the fourth year being the 'incident year', when the welfare incident was disclosed. For study herds without a clearly defined welfare incident date, the KPIs were determined on a yearly basis between 2001 and 2009. We found that the late registration of calves, the use of on-farm burial as a method of carcase disposal, an increasing number of moves to knackerries over time and records of animals moved to 'herd unknown' were notable on the case farms. Four KPIs were prominent on the case study farms and warrant further investigation in control herds to determine their potential to provide a framework for refining current systems of early warning and prevention.

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## On farm animal welfare: the utility of databases for early warning

Kelly, P.C.<sup>1</sup>, Higgins, I.<sup>2</sup>, More, S.J.<sup>2,3</sup>, Blake, M.<sup>1</sup>, Hanlon, A.<sup>3</sup>

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During the last four years, the Department of Agriculture, Food and the Marine (DAFM) has sponsored two studies to investigate on-farm animal welfare incidents that have occurred on Irish farms and that have been investigated by DAFM. The first study (unpublished) looked at incidents which occurred between September 2006 and March 2007. The more recent study on animal welfare incidents looked specifically at 18 case study herds and identified four potential key performance indicator (KPIs), the information on which was downloaded from DAFM databases, that could be used to enhance the Early Warning System (EWS), i.e. late registrations of calves, an increase in the use of on-farm burial as a method of carcase disposal, an increase in the number of carcasses sent to knackerries and animals missing from the herd profile that cannot be accounted for. That study recognised the need for these KPIs to be studied in control herds to see if they also occur in the national herd or if they are more notable in the case study herds and therefore of use in enhancing the EWS. In summary, the objective of this current study is to examine, in control herds, the four KPIs identified in the earlier DAFM study to see if their occurrence is irregular and potentially indicative of other problems occurring on the farm.

## A sociological study investigating reasons for animal welfare incidents on-farm

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In 2004, the Department of Agriculture, Food and the Marine (DAFM) in conjunction with the Farm Animal Welfare Advisory Council (FAWAC), established an Early Warning System (EWS) aimed at preventing farm animal welfare incidents taking place on Irish farms. Despite its establishment, welfare problems on Irish farms still persist. A recent DAFM sponsored study on animal welfare incidents looked specifically at 18 case study herds and identified four potential key performance indicators (KPIs) that could be used to enhance the EWS, i.e. late registrations of calves, an increase in the use of on-farm burial as a method of carcase disposal, an increase in the number of carcasses sent to knackeries and animals missing from the herd profile that cannot be accounted for. Anecdotal evidence from that study and others suggest a linkage between social factors and welfare incidents. These comprise economic, health, psychological factors, examples including the death of a parent, ill health, depression, and problems related to alcohol use. This evidence suggests that such life-events can have a direct and indirect role on individual farm management. There is no evidence of research in Ireland on linkages between social factors and animal welfare incidents. It is therefore proposed that this study will investigate and aim to identify social factors that contribute to animal welfare incidents on Irish farms. This study will take a qualitative approach, using in-depth face-to-face interviews to explore and identify these factors, and consider how they contributed to animal welfare incidents in Ireland.

## Use of farmer focus groups to evaluate a welfare scheme for suckler beef cattle

Dwane, A.M.<sup>1</sup>, More, S.J.<sup>1,2</sup>, Blake, M.<sup>3</sup>, Hanlon, A.J.<sup>1</sup>

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Schemes to improve farm animal welfare have existed in Europe since the 1990s. Reform of the Common Agricultural Policy (CAP) in 2003 provided funds for animal welfare initiatives. In 2008, Ireland's Department of Agriculture, Food and the Marine (DAFM) launched the Animal Welfare, Recording and Breeding Scheme for Suckler Herds ('Suckler Scheme'). The main aims of the scheme include enhancing welfare standards and improving genetic quality of the national beef herd. This is a voluntary scheme based on economic incentives. Initial uptake was widespread with approximately 50,000 farmers joining (approximately 76% of registered beef suckler herds). Little research has focused on the attitudes of beef farmers' to animal welfare schemes. The objectives of this study were to seek farmers' opinions of the 'Suckler Scheme', to explore the underlying reasons for these opinions as well as perceptions of the scheme's relationship to welfare, and to elicit ideas for improving future schemes. In this study, four focus groups (each comprising 7+ suckler farmers) were conducted in November 2009 in four regions of Ireland. Participants were sourced through local veterinarians and invited to attend the focus groups. Ethical approval was obtained in advance and participants received a full explanation of how data would be managed before consenting to take part. Audio recordings were transcribed verbatim and then 'coded' for topics and views mentioned. Coding and thematic analysis were carried out using NVIVO 8, a software programme designed for qualitative data analysis. The majority of participants perceived all the scheme measures as being important and relevant to good farming practices while acknowledging that not all measures related to welfare but rather to data collection and breeding of beef cattle. There was strong consensus that the measures relating to the minimum age at first calving and to meal-feeding at weaning have a positive impact on animal welfare and health. Two measures were criticized for being impractical (i.e. the amount of paperwork for recording animal events and the conditions concerning the disbudding of calves). The conditions for the timing of weaning were also criticized for having a negative financial impact at sales. Participants also suggested additional measures that could further improve animal welfare. The inability to produce high-quality beef animals at a profit is of concern to farmers. The majority anticipated that the data being collected via the scheme would help inform decisions when trying to breed a 'quality' beef animal.

## Use of farmer focus groups to explore compliance issues relating to a welfare scheme for suckler beef cattle

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On-farm animal welfare is primarily determined by farmer compliance with regulations (and quality assurance standards). The levels of compliance, however, may prove difficult to assess. Ireland's Animal Welfare, Recording and Breeding Scheme for Suckler Herds ('Suckler Scheme') was launched by the Department of Agriculture, Food and the Marine (DAFM) in 2008, providing financial incentives to suckler beef farmers for implementing a number of specified welfare practices. Initial uptake of the scheme was widespread with approximately 50,000 farmers joining (approximately 76% of eligible herds). Using the Scheme as a case study, our research has focused on exploring the factors that influence beef farmers' willingness or ability to comply with welfare guidelines. In this study, four focus groups (each comprising 7-9 farmers) were conducted in 2009. Participants were sourced through local veterinarians and invited to attend. Audio recordings of discussions were transcribed verbatim and then 'coded' for topics and views mentioned. Coding and thematic analysis were carried out using NVIVO 8. When asked to list times when welfare is most at risk, participants responded as follows: calving (88%), weaning (69%), nutrition / body condition during pregnancy (25%), handling (25%), housing (22%) and disbudding / dehorning (16%). All four groups commented that all existing scheme measures were relevant to good farming practices and therefore should continue. Participants suggested amendments to a number of measures as follows: change disbudding ages; simplify paperwork; change the training; increase scheme payments; and change weaning rules. Focus groups provide scope for deeper exploration into the attitudes and beliefs underlying participants' answers in a way that more conventional surveys may not. They also provide an opportunity for farmers to discuss compliance issues without the risk of incurring a farm inspection or financial penalty. Farmers seem motivated to comply when criteria result in financial benefit, are practical and workable, impact positively on welfare and health (e.g. minimum age at first calving and meal-feeding), or are such that failure to comply may trigger inspection and / or loss of payments (e.g. attendance at training). Farmers seem less motivated to comply when criteria seem impractical at farm level, may have a negligible or even negative effect on welfare (e.g. age for disbudding), are over-complicated (e.g. paperwork), lack consistency, or may cause financial loss (e.g. Weaning).

## Equine health and welfare

### Evaluation of current equine welfare issues in Ireland: causes, desirability, feasibility and means of raising standards

Collins, J.A.<sup>1</sup>, Hanlon, A.<sup>1</sup>, More, S.J.<sup>1,2</sup>, Wall, P.G.<sup>3</sup>, Kennedy, J.<sup>4</sup>, Duggan, V.<sup>1</sup>

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**Equine Veterinary Journal 42, 105-113 (2010)**

Significant potential threats to the health and welfare of horses exist in Ireland when supply exceeds demand and the identification system for horses is not yet robust. The objective of this study was to secure engagement with stakeholder groups and determine their perception of equine welfare in Ireland and encourage the development of inclusive, rather than imposed, policy solutions. A 3 round, web-based Policy Delphi incorporating novel vignette methodology was conducted from November 2007–March 2008 to canvass opinion (in both quantitative and qualitative forms) on the perceived most significant equine welfare issues. Vignettes (narratives depicting potential compromise to equine welfare) were employed. Quantitative data were collected in the form of scoring on a 9 point Likert scale with labelled end-points, qualitative information as text subsequently analysed for themes. All 44 respondents completed all rounds. Major equine welfare issues were identified as welfare of horses during the disposal process and at unregulated gatherings. Assessed



quantitatively on a 9 point Likert scale (0 = minimal; 8 = maximal), respondents scored the desirability and feasibility of improving standards, median 8 and 6, respectively, for both issues identified. Basic themes identified in respondents' quotes as reasons to raise equine welfare standards were ideological, protection of animal welfare, safe-guarding the reputation of the equine industry and safety (of people, horses and environment). Themes for reasons for low standards were societal norms, fiscal pressures, indolence, indifference and ignorance. Themes underpinning potential means for achieving meaningful change (solutions) were legislation, enforcement, education/training, fiscal remedies, increasing awareness and a combination of these. Mechanisms aimed at raising standards must be based on an understanding of motivational drivers for currently low standards. The challenge is to translate the findings and this heightened awareness into meaningful change to the benefit of horses and those who care for them.

*Reprinted from Equine Veterinary Journal, 42, Collins, J.A., Hanlon, A., More, S.J., Wall, P.G., Kennedy, J., Duggan, V., Evaluation of current equine welfare issues in Ireland: causes, desirability, feasibility and means of raising standards, 105-113, Copyright 2010, with permission from EVJ Ltd.*

## Case study of equine welfare on an Irish farm: 2007-2009

Collins, J.A.<sup>1</sup>, More, S.J.<sup>1,2</sup>, Hanlon, A.<sup>1</sup>, Duggan, V.<sup>1</sup>

<sup>1</sup> UCD School of Veterinary Medicine, <sup>2</sup> UCD CVERA

### **Veterinary Record 167, 90-95 (2010)**

This report describes the progression in welfare standards for horses on a horse farm in the Republic of Ireland between 2007 and 2009. Visits to the farm were undertaken and information, in the form of written notes and digital recording of observations and examinations, was gathered in consultation with officials from the Gardaí (the Irish police), the Department of Agriculture, Food and the Marine and the Irish Society for the Prevention of Cruelty to Animals. Further independent veterinary corroboration of clinical findings and laboratory support occurred following seizure of the horses. The complex reality of on-farm equine welfare problems and the difficulties in achieving a resolution are discussed compared with other species conventionally considered to be food-producing animals.

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## Aspects of the owning/keeping and disposal of horses, and how these relate to equine health/welfare in Ireland

Collins, J.A.<sup>1</sup>, Hanlon, A.<sup>1</sup>, More, S.J.<sup>1, 2</sup>, Wall, P.G.<sup>3</sup>, Duggan, V.<sup>1</sup>

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**Irish Veterinary Journal 64, 11 (2011)**

Ireland has long been renowned as a major centre for the breeding, rearing and keeping of horses. Since 2007, however, there has been increasing concern for horse health and welfare standards, and links between these concerns and the structures, governance and funding of the Irish equine industries have been reported. This paper addresses two central issues: firstly, the local governance of, trade in and disposal of unwanted horses; and secondly, mechanisms employed to improve standards of care given to horses owned by certain communities. Primary information was gathered through visits to horse pounds run by, and on behalf of, Local Authorities, to social horse projects, to horse dealer yards, ferry ports, horse slaughter plants and knackerries. The approach adopted by members of a given group, e.g. ferry ports, is described and differences are highlighted, for example in how different Local Authorities implement the Control of Horses Act of 1986, and how the choice, for example, of disposal route affects the standard of animal welfare. There is a pressing need for a more centrally mandated and uniformly applied system of governance to safeguard the health and promote the keeping of horses to a higher welfare standard in Ireland. Fundamental to an understanding of why there is insufficient oversight of the keeping and proper disposal of horses is the lack of a comprehensive, integrated system for the registration, identification and tracing of equidae in Ireland.

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## Use of qualitative methods to identify solutions to selected equine welfare problems in Ireland

Collins, J.A.<sup>1</sup>, More, S.J.<sup>2, 3</sup>, Hanlon, A.<sup>3</sup>, Wall, P.G.<sup>4</sup>, McKenzie, K.<sup>5</sup>, Duggan, V.<sup>3</sup>

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**Veterinary Record (in press)**

This paper explores the views of those in the Irish equine industry, organisations and government regarding necessary improvements to equine welfare in Ireland at unregulated gatherings and during the disposal process. Three qualitative research methods were employed, namely semistructured interviews, focus groups and a structured, facilitated workshop. Representatives from industry, welfare societies, socially disadvantaged groupings and government engaged with this process and shared their views regarding horse welfare and implementable solutions with merit to address welfare problems. A consensus was achieved that equine welfare in Ireland could be improved by the development of a comprehensive identification system, a Code of Practice for horse gatherings, a horse licensing scheme, ring-fenced funding to promote responsible, humane horse disposal and better means of raising awareness of the value of safeguarding horse welfare for the benefit of all parties.

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## The development and application of a tool to assess the welfare of equidae at fairs and markets in Ireland

Collins, J.A.<sup>1</sup>, Johnson, J.<sup>1</sup>, Hanlon, A.<sup>1</sup>, More, S.J.<sup>1,2</sup>, Duggan, V.<sup>1</sup>

<sup>1</sup> UCD School of Veterinary Medicine, <sup>2</sup> UCD CVERA

Unregulated horse gatherings, such as fairs and markets, are considered to be of high value culturally, socially and economically to Ireland. However, a Delphi study completed in 2008 by Collins *et al.* identified welfare standards for horses at such events as one of the two most significant equine welfare concerns in Ireland. In this paper, we describe the development of an equine welfare assessment protocol, based on the Five Freedoms, which employs measurement of both welfare inputs and outputs, and early attempts to apply this protocol at fairs and markets in Ireland. Thirty parameters (based on a semi-qualitative 5 point Likert scale) were each measured on thirty occasions alongside details of weather, horse numbers and the presence of an organising committee. The dataset ( $n=30$ ) was divided into three subsets (with some overlap) to enable the analyses of welfare scores for individual events ( $n=14$ ), inter-observer variability in scores at events scored simultaneously but independently by the first two authors, JC and JJ ( $n=5$ ), and change over time at any one event ( $n=8$ ). By the criterion of importance adopted by the authors, the freedom of horses to access water, feed and shelter (resource input) and the ability of horses to drink and feed (current output) were deemed to be unduly compromised. The presence of an organising committee was not found to be protective of equine welfare. It is suggested by the authors that a combined approach (input and output-based measures) to the assessment of equine welfare at unregulated gatherings be adopted but that further work is required to refine the protocol to ensure that repeatability and reproducibility of scoring are achieved in its implementation.

## Development of a bio-security assessment tool and its application at equine events in Ireland

Johnson, J.P.<sup>1</sup>, More, S.J.<sup>1,2</sup>, Collins, J.A.<sup>3</sup>, Duggan, W.E.<sup>1</sup>

<sup>1</sup> UCD School of Veterinary Medicine, <sup>2</sup> UCD CVERA, <sup>3</sup> Norbrook Laboratories Ltd (Ireland), Newry, Co. Down, Northern Ireland

There is the potential for disease transmission wherever horses gather in groups. The inconsistent application of disease control measures across the horse industries in Ireland, the highly contagious nature of many of the equine infectious diseases, the potential for the spread of disease from sub-clinically infected horses and increased transport of horses to international events together mean that all horses are put at risk of disease transmission, particularly in the event of an outbreak of an exotic equine disease. The objectives of this study were to develop a bio-security assessment tool for use at equine events, to apply the tool in the investigation of the potential for contagious disease transmission at equine events in Ireland, and to determine the influence of the degree of regulation of event on risk of disease transmission. A scoring system was developed to identify bio-security risks at equine events. This was based on both direct and indirect risk factors which contribute to contagious disease transmission. Risk factors included categories such as contact between horses, contact with fomites, feeding facilities, degree of public access, control of wildlife, sanitation of stables and housing ventilation. The regulation status of each event was determined based on a combination of published governing rules and observation of their enforcement during on-site visits. Highly regulated events had significantly lower overall risk levels than partially regulated events ( $p<0.003$ ); overall risk level of partially regulated events did not differ significantly from that of unregulated events ( $p=0.051$ ).

## A retrospective study of horses investigated for weight loss despite a good appetite (2002-2011)

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<sup>1</sup> UCD School of Veterinary Medicine, <sup>2</sup> UCD CVERA

Weight loss despite a good appetite is a frequent diagnostic challenge for equine veterinarians; however, there are few objective reports and little descriptive information regarding risk factors and prognostic indicators. The aim of this study was evaluate the relationship between historical and clinicopathological findings and final outcome in order to identify risk factors and prognostic indicators. Medical records of horses referred for investigation of weight loss despite a good appetite were reviewed. Data collated included history, signalment, clinical and diagnostic findings, diagnoses and outcome (survival vs non-survival). Univariable associations were evaluated with a Mann-Whitney *U* test, Fisher's Exact test (categorical or binary data) or Pearson's rank correlation (continuous data), with  $P \leq 0.05$  considered significant. Forty cases met the inclusion criteria. Albumin concentrations at admission were significantly ( $P = 0.008$ ) higher in survivors ( $29.1 \pm 6.5$  g/L) than non-survivors ( $22.8 \pm 5.4$  g/L) and positively correlated with outcome ( $r^2 = 0.23$ ;  $P = 0.005$ ). Animals with low total protein ( $P = 0.029$ , OR = 7, 95 % CI = 1.22 – 40.1) and albumin ( $P = 0.032$ , OR = 6, 95% CI = 1.22 – 29.5) concentrations were at greater risk for non-survival. Body condition score was positively correlated with total protein ( $r^2 = 0.17$ ;  $P = 0.03$ ) and albumin ( $r^2 = 0.53$ ;  $P < 0.0001$ ) concentrations at admission and duration of clinical signs ( $r^2 = 0.19$ ;  $P = 0.03$ ). The severity of hypoproteinaemia and hypoalbuminaemia are related with a worsening prognosis. Since body condition score is positively associated with albumin concentration, it could potentially be used as a prognostic indicator for survival in conjunction with albumin concentration.

## Porcine welfare

### The use of meat inspection as a surveillance tool for improved pig welfare

Harley, S.<sup>1</sup>, O'Connell, N.<sup>2</sup>, Boyle, L.<sup>3</sup>, More, S.J.<sup>1,4</sup>, Hanlon, A.<sup>1</sup>

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Food animal welfare is synonymous with food quality and linked to food safety. This link is evident at slaughter, when ante-mortem and post-mortem inspections are conducted to ensure that the animal is fit for human consumption, carcasses that are damaged as a result of bruising, lesions, disease and injury are trimmed or rejected, depending on the extent of disease and damage. Damage to carcasses is likely to be indicative of the housing, and management of the animal during the production period, and thus reflect the animals welfare. Disease and injury such as lesions and bruising are undeniably an indication of poor welfare. The main objectives of this research is to explore the use of carcass condemnation as a surveillance tool for farm animal welfare and to examine the process of carcass condemnation of swine in Ireland, including the reporting mechanisms and the economic losses to the farmer and the processor.

## Emergency issues

A qualitative assessment of the risk of spread to and within the commercial poultry industry, following the introduction of H5N1 avian influenza into Ireland, and of potential risk mitigation measures

Aznar, I.<sup>1</sup>, Crowe, O.<sup>2</sup>, Wilson, J.<sup>3</sup>, Duignan, P.J.<sup>4</sup>, Gaynor, S.<sup>4</sup>, Neilan, R.<sup>4</sup>, McLoon, D.<sup>5</sup>, McArdle, P.J.<sup>4</sup>, More, S.J.<sup>1,6</sup>

<sup>1</sup> UCD CVERA, <sup>2</sup> Birdwatch Ireland, <sup>3</sup> National Parks and Wildlife Service, <sup>4</sup> DAFM, <sup>5</sup> Private practitioner, <sup>6</sup> UCD School of Veterinary Medicine

This paper describes work conducted in 2008 to qualitatively assess the risk of spread to and within the commercial poultry industry, following the introduction of H5N1 avian influenza into Ireland, and of potential risk mitigation measures. Four transmission routes were considered, including spread among wild waterbirds, spread from wild waterbirds to non-commercial avian operations, spread from wild waterbirds to commercial poultry (directly or via non-commercial avian operations) and spread within the commercial poultry industry. There is considerable potential in Ireland for spread of H5N1 avian influenza among wild waterbirds, and from wild waterbirds to non-commercial avian operations. In contrast, the opportunity for spread to and within the commercial poultry sector is variable, depending on a broad range of factors including production system, water source and management. A number of important risk mitigation measures were identified, focusing on improved information and awareness, risk based surveillance and maintenance of up-to-date databases. Each of these issues has now been considered in detail, both by DAFM and industry.

## An outbreak of piroplasmosis in horses in Ireland

Bailey, D.<sup>1</sup>, Leadon, D.L.<sup>2</sup>, Brangan, P.<sup>1</sup>, de Waal, T.<sup>3</sup>, Lenihan, P.<sup>4</sup>, Larkin, J.<sup>1</sup>, Ennett, F.<sup>1</sup>, Gaynor, S.<sup>1</sup>, More, S.J.<sup>3,5</sup>

<sup>1</sup> DAFM, <sup>2</sup> Irish Equine Centre, <sup>3</sup> UCD School of Veterinary Medicine, <sup>4</sup> DAFM Veterinary Laboratory Service, <sup>5</sup> UCD CVERA

An outbreak of piroplasmosis (caused by *Theileria equi*) occurred in horses in Ireland during 2009. The objective of this paper is to describe the outbreak, the transmission of *T. equi* within Ireland and aspects of outbreak management.

## Outbreak of bovine brucellosis in County Clare, Ireland, during 2005

Hayes, M.<sup>1</sup>, Kilroy, A.<sup>1</sup>, Ashe, S.<sup>1</sup>, Power, S.<sup>2</sup>, Kenny, K.<sup>2</sup>, Collins, D.M.<sup>3</sup>, More, S.J.<sup>3,4</sup>

<sup>1</sup> DAFM, <sup>2</sup> DAFM Veterinary Laboratory Service, <sup>3</sup> UCD CVERA, <sup>4</sup> UCD School of Veterinary Medicine

### Veterinary Record 166, 107-111 (2010)

This paper describes an investigation of an outbreak of bovine brucellosis in County Clare, Ireland, during 2005. It is likely that infection on the index farm was linked to a previous outbreak of brucellosis in County Clare. During March to May 2005, transmission of brucellosis within the herd was rapid; this was facilitated by a range of factors, including close contact between cattle kept in winter housing, and the mixing of animals, both during grazing and at housing, throughout the year. Containment of the disease, including only limited spread to one contiguous herd, was facilitated by the recent construction of a shed for winter housing.

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## Transmissible spongiform encephalopathy (TSE)

### Factors contributing to sample quality for the BSE active surveillance programme in the Republic of Ireland

Cahill, A.<sup>1</sup>, Collins, D.M.<sup>2</sup>, Aznar, I.<sup>2</sup>, More, S.J.<sup>2,3</sup>, Griffin, J.<sup>1</sup>, Sheridan, H.<sup>1</sup>

<sup>1</sup> DAFM, <sup>2</sup> UCD CVERA, <sup>3</sup> UCD School of Veterinary Medicine

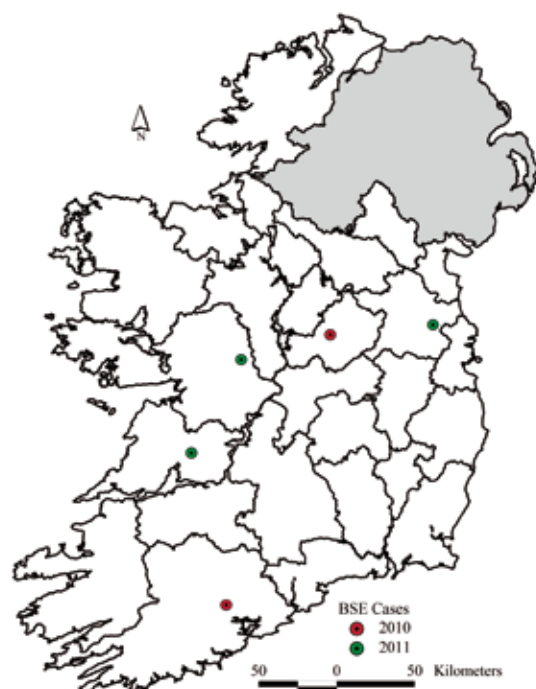
The Republic of Ireland's active surveillance programme for BSE involves the collection of brain stem tissue samples from certain categories of cattle, including cattle which die on farm (fallen cattle). Some of these samples are of poor quality due to autolysis and this has implications for accurate BSE diagnosis. The Republic of Ireland is obliged under EU law to take measures to minimise sample autolysis. Data on the degree of autolysis of all samples taken between 2007 and 2011 have been captured on the AHCS (Animal Health Computer System). The objective of this study is to identify risk factors for poor sample quality in fallen animals in the Republic of Ireland.

### Bovine spongiform encephalopathy in the Republic of Ireland before and after the reinforced feed ban: epidemiology, spatial analysis and risk factors

Ryan, E.<sup>1</sup>, McGrath, G.<sup>2</sup>, Sheridan, H.<sup>3</sup>, More, S.J.<sup>2,4</sup>, Aznar, I.<sup>2</sup>

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Bovine spongiform encephalopathy (BSE) is a prion disease of cattle, spread by contamination of cattle feed. In the Republic of Ireland, a reinforced feed ban on mammalian meat and bone meal (MBM) was introduced on 1st October 1996 to stop further infection of cattle. Between then and July 2010, 44 cases of BSE from 40 herds have been born, termed "born after the reinforced ban" or "BARB" BSE cases. The objectives of this project were to describe the epidemiology of these BARB cases, to determine area-level risk factors for BSE and how they related to the stage of the BSE epidemic, and to evaluate whether the spatial pattern of BSE cases was non-random and had changed over time.



*Confirmed BSE cases in Ireland during 2010 & 2011.*

## Q fever

### Q fever in humans and farmed animals in four European countries

Georgiev, M.<sup>1</sup>, Afonso, A.<sup>1</sup>, Neubauer, S.<sup>2</sup>, Needham, H.<sup>3</sup>, Thiery, R.<sup>4</sup>, Rodolakis, A.<sup>5</sup>, Roest, H.J.<sup>6</sup>, Staerk, K.<sup>7</sup>, Stegeman, J.A.<sup>8</sup>, Vellema, P.<sup>9</sup>, van der Hoek, W.<sup>10</sup>, More, S.J.<sup>11, 12</sup>

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Q fever, is a zoonotic bacterial disease in humans, caused by *C. burnetii*, and a large range of animals can be infected. This paper presents a review of Q fever and *C. burnetii* infection in humans and farmed animals, using case studies from four European countries. In all four countries, the serological prevalence of *C. burnetii* infection and reported incidence of Q fever varies broadly in both farmed animals and humans. Animal proximity and contact with infected animals or their birth products have been identified as the most important risk factors for human disease. Intrinsic farm factors, such as production system and management, influence the number of outbreaks in an area. Potential control options are common methods of disease control have included measures to increase diagnostic precision and general awareness, and to reduce human exposure and spill over. This study highlights important gaps in knowledge, and future research needs.

### Seroprevalence of *Coxiella burnetii* antibodies in sheep and goats in the Republic of Ireland

Ryan, E.<sup>1</sup>, Kirby, M.<sup>1</sup>, Clegg, T.<sup>2</sup>, Collins, D.M.<sup>2</sup>

<sup>1</sup> DAFM Veterinary Laboratory Service, <sup>2</sup> UCD CVERA

#### Veterinary Record 169, 280 (2011)

Q fever is a zoonotic disease caused by *Coxiella burnetii*, a bacteria whose main reservoirs are goats, sheep and cattle but which can also infect a wide range of mammals. The objective of this study was to estimate the prevalence of exposure to *C. burnetii* in sheep and goats in the Republic of Ireland. Serum from 2,197 sheep from 119 flocks and 590 goats from 66 herds, randomly sampled, were tested for the presence of *C. burnetii* antibodies using an indirect ELISA. In sheep, 15/2,197 (0.7 per cent) samples from 10/119 (8.4 per cent) flocks were positive. In goats, 2/590 (0.3 per cent) samples from 1/66 (1.5 per cent) herd were positive. The results confirm the exposure of sheep and goats to *C. burnetii* in the Republic of Ireland.

*Adapted from Veterinary Record, Ryan, E., Kirby, M., Clegg, T., Collins, D.M., 169, 280, Copyright 2011, with permission from BMJ Publishing Group Ltd.*

## Prevalence of *Coxiella burnetii* (Q fever) antibodies in bovine serum and bulk-milk samples

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### Epidemiology and Infection 139, 1413-1417 (2011)

Q fever (*Coxiella burnetii*) is a zoonotic disease of increasing public health importance. The objective of this study was to estimate the prevalence of, and risk factors associated with, exposure to *C. burnetii* in cattle in the Republic of Ireland. Bulk-tank milk samples from 290 dairy herds and 1,659 sera from 332 dairy and beef herds, randomly sampled, were tested by indirect ELISA to detect antibodies to *C. burnetii*. In total, 37.9 % of bulk-milk sample herds and 1.8% of sera (from 6.9% of herds) were antibody positive. Of risk factors tested using logistic regression analysis, only large herd size (bulk-milk analysis) and dairy breed (serum analysis) significantly increased the odds of being positive for antibodies to *C. burnetii*. Herds with positive milk or serum samples were randomly distributed throughout the Republic of Ireland and no clustering was observed. The use of an ELISA to test bulk-milk samples collected by randomized stratified sampling is a cost-effective method by which national herd prevalence can be estimated by active surveillance.

*Reprinted from Epidemiology and Infection, 139, Ryan, E.D., Kirby, M., Collins, D.M., Sayers, R., Mee, J.F., Clegg, T., Prevalence of Coxiella burnetii (Q fever) antibodies in bovine serum and bulk-milk samples, 1413-1417, Copyright 2011, with permission from Cambridge University Press.*



*Keem beach, Achill Island. Photograph by E. Gormley.*

## Companion animal epidemiology

### The spatial distribution of pet dogs and pet cats on the island of Ireland

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**BMC Veterinary Research 7, 28 (2011)**

There is considerable international research regarding the link between human demographics and pet ownership. In several international studies, pet ownership was associated with household demographics including: the presence of children in the household, urban/rural location, level of education and age/family structure. What is lacking across all these studies, however, is an understanding of how these pets are spatially distributed throughout the regions under study. This paper describes the spatial distribution of pet dog and pet cat owning households on the island of Ireland. In 2006, there were an estimated 640,620 pet dog owning households and 215,542 pet cat owning households in Ireland. These estimates are derived from logistic regression modelling, based on household composition to determine pet dog ownership and the type of house to determine pet cat ownership. Results are presented using choropleth maps. There is a higher density of pet dog owning households in the east of Ireland and in the cities than the west of Ireland and rural areas. However, in urban districts there are a lower proportion of households owning pet dogs than in rural districts. There are more households with cats in the urban areas, but the proportion of households with cats is greater in rural areas. The difference in spatial distribution of dog ownership is a reflection of a generally higher density of households in the east of Ireland and in major cities. The higher proportion of ownership in the west is understandable given the higher proportion of farmers and rural dwellings in this area. Spatial representation allows us to visualise the impact of human household distribution on the density of both pet dogs and pet cats on the island of Ireland. This information can be used when analysing risk of disease spread, for market research and for instigating veterinary care.

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### Companion animal ownership: understanding the attitudes, beliefs and behaviour of pet owners towards their pets

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Pet owners own pets for a variety of reasons including companionship, protection, and as working animals. Ownership is also a contributor to owners' physical and mental health. Some studies have been conducted on issues relating to ownership, including pet neutering and obesity. As yet, little data are available from qualitative research. In this study, we investigate pet owners' attitudes, beliefs and behaviours towards their pets, with specific emphasis on, diet, weight control and exercise. An interview-administered survey questionnaire and focus group discussions were conducted for the study. Data was coded and managed using Nvivo 8 qualitative data analysis software and StataSE Version 10<sup>®</sup>. Several themes for owning a pet emerged in the study, most commonly associated with companionship and child development. Owners tend to spoil their pet(s) with food despite being aware that this could lead to their pet becoming overweight. Owners believe that cats exercise themselves, whereas dogs need to be walked off the lead to exercise properly. Having a pet was considered the equivalent to having an extra person in the family. Our results help provide information on how owners and their pets may benefit from tailored counselling about pet exercise and neutering.



## Neutering in Ireland: Investigating risk factors for neutering and examining owner attitudes and beliefs towards neutering

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Companion animal over-population is an old problem which causes significant costs to humans and governments every year (Olson *et al.* 1991). Over-population has been associated with owners being unconcerned about neutering their pets or allowing them to stray (Soto *et al.* 2005; Natoli *et al.* 2006; Weng *et al.* 2006). Cat owners are more likely to neuter than dog owners (Franti *et al.* 1980; Leslie *et al.* 1994; Poss and Bader 2007; Downes *et al.* 2009; Faver 2009; McKay *et al.* 2009). In this study, we investigate risk factors for neutering a pet and examine pet owners' attitudes, beliefs and behaviours towards their pets, with specific emphasis on neutering. Data from 3 sources were included in this study; a computer assisted telephone interview, one to one interviews and focus group discussions conducted specifically for the study. Data was coded and managed using Nvivo 8 qualitative data analysis software and StataSE Version 10<sup>®</sup>. Reasons for neutering included, preventing unwanted pregnancies, to limit wandering behaviour and prevent disease. Individual pet characteristics played a role in the decision of owners to neuter their pet(s). Some owners suggested that they would be reluctant to neuter again after past experience.

## Factors associated with furry pet ownership among patients with asthma

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**Journal of Asthma 47, 742-749 (2010)**

Exposure to indoor allergens is an established risk factor for poor asthma control. Current guidelines recommend removing pets from the home of patients with asthma. This cross-sectional study was conducted to determine the prevalence of furry pet ownership in asthmatics compared to non-asthmatics and to identify factors associated with furry pet ownership among those with asthma. Secondary analysis assessed characteristics among asthmatics that might be associated with allowing a furry pet into the bedroom. Using data from The National Asthma Survey collected from 2003 to 2004, we carried out univariate and multiple regression analyses, in 2009, to identify independent predictors of furry pet ownership in asthma sufferers after controlling for potential confounders. Overall, asthmatics were more likely to own a furry pet than nonasthmatic individuals in the general population (49.9% versus 44.8%,  $p < .001$ ). Multivariate analysis showed that female sex, older age, white race, and high income were independent predictors of furry pet ownership among asthmatics. Additionally, 68.7% of patients with asthma who own a furry pet allowed them into their bedroom. Higher income and carrying out  $\leq 2$  environmental control practices in the home were associated with increased likelihood of allowing a furry pet into the bedroom. Furry pet ownership is equally or more common among asthmatics compared to those without asthma. The majority of asthmatics with furry pets allow them into the bedroom. Recognizing and addressing these problems may help decrease asthma morbidity.

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## Cadmium exposure in cattle

### National survey of cadmium in bovine kidneys

Canty, M.J.<sup>1</sup>, Collins, D.M.<sup>1</sup>, Scanlon, A.<sup>2</sup>, More, S.J.<sup>1,3</sup>, Sheridan, M.<sup>2</sup>

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Of all the animal tissues, livers and kidney constitute a special dilemma in that they have a tendency to bioaccumulate toxic metals such as lead (Pb), cadmium (Cd), mercury (Hg) and arsenic (As). Maximum levels (ML) for Hg, Pb, Cd and tin (Sn) in foodstuffs are set by Commission Regulation No. 1881/2006 (amended by No. 629/2008), which includes bovine muscle, livers and kidneys. Cadmium, a widely distributed environmental industrial pollutant, has no beneficial biological role and may be highly toxic when introduced into the body by ingestion or inhalation to both animals and humans. Cadmium is not easily cleared by the cells and the poor efficiency of cellular export systems explains the long residence time of this element in storage tissues such as the intestine, the liver and the kidneys, resulting in older animals having higher liver and kidney Cd concentrations even if the levels in their diets and water are consistently low. Cadmium has no known biological function in either animals or humans but mimics the actions of other divalent metals that are essential to diverse biological functions. Cadmium bioavailability, retention and consequently toxicity are affected by several factors such as nutritional status (low body iron stores) and multiple pregnancies, pre-existing health conditions or diseases. The objective of this study is to determine cadmium concentrations in bovine kidneys randomly collected from cattle over five years of age from the 26 counties of Ireland.

### Cadmium review

Lane, E.A.<sup>1</sup>, Canty, M.J.<sup>1</sup>

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No biological role has been described for cadmium (Cd) in animals and its presence in animal tissue is considered unnecessary. Cadmium is considered to be one of the most toxic substances in the environment due to its wide range of organ toxicity and long elimination half-life. Batteries are an important source of Cd pollution, additionally, combustion of coal, smelting, mining, alloy processing and industries that employ Cd as a dye are also potential sources of Cd pollution. Agricultural practices such as the application of sewage sludge and contaminated fertilizers are also sources of Cd contamination. Absorption of Cd occurs via the respiratory and digestive system. Approximately 10 to 50% of Cd fumes are absorbed by the respiratory system. While, Cd is poorly absorbed via the digestive tract, compared to similar divalent cations, Zn and Fe; approximately 5% of oral Cd is absorbed. Once absorbed, Cd circulates in red blood cells or bound to albumin in plasma. Cadmium interacts with the metabolism of essential minerals; calcium, zinc, iron, copper and selenium. The majority of newborn ruminants have a low Cd burden. Accumulation occurs slowly over time, primarily in liver and kidneys. In the liver it may induce and bind metallothionein, this complex is released slowly into circulation and then accumulates in kidneys. At high levels, dietary Cd can cause decreased feed intake, and lowered weight gain, anaemia, decreased bone absorption and abortions and Cd toxicity has been reported in many species including cattle.

## International collaboration

### Quantitative Rose Bengal test for diagnosis of bovine brucellosis

Cho, D.<sup>1</sup>, Nam, H.<sup>1</sup>, Kim, J.<sup>1</sup>, Heo, E.<sup>1</sup>, Cho, Y.<sup>1</sup>, Hwang, I.<sup>1</sup>, Kim, J.<sup>1</sup>, Kim, J.<sup>1</sup>, Jung, S.<sup>1</sup>, More, S.<sup>2,3</sup>

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**Journal of Immunoassay and Immunochemistry 31, 120-130 (2010)**

The Rose Bengal Test (RBT) is the most widely used screening test for brucellosis in both humans and animals. Owing to its apparent simplicity of reading, however, interpretations of the RBT results can be affected by personal experience. This study describes a simple way to improve the accuracy and uniformity of reading the RBT reaction by counting the number of agglutinated particles using transparent OHP film with Quantity One®, which was originally designed to count the bacterial colony numbers on agar plates. Using this system, the reactivities of three Rose Bengal antigens from different sources against international standard serum (1,000 units, VLA, UK) could be numerically measured: the intensity scale ranged from zero to around 1,600. This system enabled us to compare the antigenicity of Rose Bengal antigens from three different sources by using statistical analyses such as regression and mean intensity. Collectively, mathematical measuring of the reaction intensity used in this study may help interpret subtle test results by providing more reliable data and additional statistical information on the herd. In addition, the method would also be applicable to other agglutination test for other diseases.

*Reprinted from Journal of Immunoassay and Immunochemistry, 31, Cho, D., Nam, H., Kim, J., Heo, E., Cho, Y., Hwang, I., Kim, J., Kim, J., Jung, S., More, S., Quantitative Rose Bengal test for diagnosis of bovine brucellosis, 120-130, Copyright 2010, with permission from Taylor and Francis Ltd.*

### Epidemiological characteristics of bovine brucellosis in Korea, 2001-2004

Nam, H.-M.<sup>1</sup>, Kim, C.-H.<sup>1</sup>, More, S.J.<sup>2,3</sup>, Yoon, H.<sup>1</sup>, Kim, S.J.<sup>1</sup>, Lee, B.-Y.<sup>1</sup>, Park, C.-K.<sup>1</sup>, Jeon, J.-M.<sup>1</sup>, Wee, S.-H.<sup>1</sup>

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This paper describes the epidemiological characteristics of bovine brucellosis in Korea during January 2000-September 2004, which encompasses the period when the incidence of bovine brucellosis increased abruptly. Data from the National Animal Infectious Disease Data Management System were used for this study. A range of epidemiological measures were calculated including annual herd and animal incidence. During the study period, there were 1,183 outbreaks on 638 farms. In beef cattle, annual herd incidence increased from 0.15 (2000) to 11.5 (2004 to September) outbreaks per 10,000 and annual animal incidence varied between 3.4 (2000) and 105.8 (2004, to September) per 100,000, respectively. On 401 (62.9%) farms during this period, infection was eradicated without recurrence. Recurrence of infection was significantly higher on farms where abortion was reported (53.3%) compared to farms where it wasn't (30.0%). On beef cattle farms, infection was introduced most frequently through purchased cattle (46.2%). Based on the results of this study, the establishment and spread of brucellosis in the Korean beef cattle population was mainly due to incomplete or inappropriate treatment of aborted material and the movement of infected cattle.

## *Mycobacterium bovis* in Korea: an update

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### **The Veterinary Journal 185, 347-350 (2010)**

This paper reports changes in the cattle population and the incidence of bovine tuberculosis in the Republic of Korea between 1960 and 2007, and discusses potential factors contributing to the recently observed increase in disease incidence, particularly in beef cattle and deer. Although there have been ongoing refinements to the existing programme, further improvements in current strategies are needed, including surveillance of susceptible animal species, both domestic and wild, and ongoing surveillance of the human population.

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## An outbreak of highly pathogenic avian influenza at a public animal exhibit in Seoul, Korea during 2008

Yoon, H.<sup>1</sup>, Moon, O.K.<sup>1</sup>, More, S.J.<sup>2, 3</sup>, Park, C.K.<sup>4</sup>, Park, J.Y.<sup>1</sup>, Lee, Y.J.<sup>1</sup>, Lee, S.D.<sup>1</sup>, Ha, J.K.<sup>1</sup>, Jeong, S.K.<sup>1</sup>, Jeong, J.W.<sup>1</sup>, Lee, S.J.<sup>1</sup>

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### **Zoonoses and Public Health 57, 142-145 (2010)**

This study describes the first recorded outbreak of HPAI in the city of Seoul, in captive birds held in an exhibition for public viewing at a local district office. The index cases were two pheasants, which had been introduced into the exhibit on 24 April, 4 days prior to death, from a store in a local market in Gyeonggi-do. Ducks and chickens from an HPAI outbreak farm, subsequently confirmed on 4 May, had also been held in this store. This outbreak highlights the potential role of local markets in AIV transmission. This outbreak led to considerable public health concern in Korea, however, no human cases were reported. The non-commercial poultry sector needs to be considered in national plans for preparedness and response.

*Reprinted from Zoonoses and Public Health, 57, Yoon, H., Moon, O.K., More, S.J., Park, C.K., Park, J.Y., Lee, Y.J., Lee, S.D., Ha, J.K., Jeong, S.K., Jeong, J.W., Lee, S.J., An outbreak of highly pathogenic avian influenza at a public animal exhibit in Seoul, Korea during 2008, 142-145, Copyright 2010, National Veterinary Research and Quarantine Service.*

## Miscellaneous

### Endophyte review/opinion paper

Canty, M.J.<sup>1</sup>

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Animals grazing or consuming conserved forages can encounter problems if forages are infected with an endophyte, which produces toxins which are harmful to animals. Endophytes are fungi that live within a plant and the relationship between a grass and its endophyte is symbiotic. The grass provides the nutrients and the endophyte produces toxins that fend off insects, diseases and grazing animals. Endophytes are transmitted only through grass seed. Different species of endophyte fungus infect different species of grass. Principally we are concerned with the fungus *Neotyphodium coenophialum*, which produces over 32 toxic alkaloids including 17 ergot alkaloids that affect livestock. The principal toxin is ergovaline, which amongst other alkaloids, causes fescue toxicosis in livestock. These toxins are vasoconstrictors, they constrict blood vessels and reduce circulation to the body extremities, interfering with the animals ability to regulate body temperature and causing conditions called 'fescue foot' in cold weather and 'summer slump' in hot weather. The objective of this study is to determine the role of the endophyte ergovaline on a number of farms where poor animal performance had been reported.

### Graduate Certificate in Dairy Herd Health

Cashman, D.<sup>1</sup>, Williams, E.J.<sup>1</sup>, Doherty, M.L.<sup>1</sup>, More, S.J.<sup>1, 2</sup>, Mulligan, F.<sup>1</sup>

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Online learning is growing in popularity as a method to deliver lifelong learning to veterinary professionals, as many are unable to commit to full-time, on-campus educational programmes. The number of academic staff engaging in this method of educational delivery is low, however. There are challenges to the development and delivery of high-quality online learning programmes, recent reductions in university resources, both human and financial. The Dairy Herd Health group in the University College of Dublin, aimed to overcome these hurdles, and set out to develop and deliver an online graduate certificate in dairy herd health. Targeted at veterinarians out in practice, practitioners will be equipped with tools for herd data analysis, practical herd evaluation skills, and an ability to integrate the multiple facets of dairy herd health for the development of holistic herd level solutions that are set in context with regard to herd profitability and animal health and welfare. The Certificate was successfully delivered in 2011. This paper offers a framework for the development of online graduate programmes with limited resources and novice academic staff new to online delivery of programmes. We reflect on the challenges that academic staff members encountered in the design and delivery of their online teaching strategies. Finally we provide recommendations on how to overcome these teaching and learning challenges.

## Preface. SVEPM 2010 – The role of veterinary epidemiology in animal health in the world today

Martinez, T.A.<sup>1</sup>, Pfeiffer, D.U.<sup>2</sup>, More, S.J.<sup>3, 4</sup>

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### Preventive Veterinary Medicine 100, 89 (2011)

This preface provides an introduction to a special edition of Preventive Veterinary Medicine, based on research presented at the 2010 Society of Veterinary Epidemiology and Preventive Medicine conference. The papers range from classical approaches of observational epidemiology applied to current problems, to more novel techniques involving contact structure and social network analysis, and culminate with an introduction to advanced statistical methods of analysing Bayesian networks.

*Adapted from Preventive Veterinary Medicine, 100, Martinez, T.A., Pfeiffer, D.U., More, S.J., Preface. SVEPM 2010 – The role of veterinary epidemiology in animal health in the world today, 89, Copyright 2011, with permission from Elsevier.*

## Evidence is at the core of scientific method: A challenge for clinicians

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### The Veterinary Journal (in press)

Evidence is at the core of the scientific method, providing the basis for acquiring new and correcting and consolidating existing knowledge. Within the disciplines of veterinary medicine, evidence is generally gathered through experimentation and/or observation, with conclusions then being drawn using inductive and/or deductive reasoning. There are many issues relating to scientific evidence, although only two are considered in this paper. Firstly, scientific evidence needs to be presented to end-users, including clinicians, policy-makers and other scientific researchers, in a manner that facilitates critical evaluation, hence the importance of internationally reporting guidelines. Secondly, scientific evidence, combined with clinical judgement and expertise, forms the foundation for evidence-based veterinary medicine (EBVM). This guest editorial provides an introduction to a separate critique of EBVM, including strategies to increase its adoption in clinical practice.

*Adapted from The Veterinary Journal, 191, More, S.J., Evidence is at the core of scientific method: A challenge for clinicians, 11-12, Copyright 2012, with permission from Elsevier.*

## Improving the quality of reporting in veterinary journals: how far do we need to go with reporting guidelines?

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### The Veterinary Journal 184, 249-250 (2010)

Publication in the international peer-reviewed literature is one of the most important outputs of any research. Despite its importance, however, the quality of reporting is variable. Guidelines have been developed by international scientific



teams to promote the quality of reporting of research studies, thereby improving both the value and reliability of medical research literature. Increasingly, key medical journals, either require or recommend author compliance with reporting guidelines. As yet, however, a similar approach is not standard practice among veterinary journals. This would be partly resolved through efforts to raise awareness of these reporting guidelines among veterinary researchers. In addition, veterinary journals should require author compliance with relevant reporting guidelines, in the interest of high quality reporting of veterinary medical research.

*Adapted from The Veterinary Journal, 184, More, S.J., Improving the quality of reporting in veterinary journals: how far do we need to go with reporting guidelines?, 249-250, Copyright 2010, with permission from Elsevier.*

## Markers of the uterine innate immune response of the mare

Nash, D.M.<sup>1</sup>, Sheldon, I.M.<sup>1,2</sup>, Herath, S.<sup>1,3</sup>, Lane, E.A.<sup>4</sup>

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### Animal Reproduction Science 119, 31–39 (2010)

Reproductive efficiency in mares is low and persistent mating-induced endometritis (PMIE) is an important cause of subfertility. Mating-induced endometritis (MIE) an obligate precursor to PMIE, is a ubiquitous, transient inflammatory response to the presence of sperm, seminal components and pathogens. However, the specific inflammatory pathways that derive from MIE and that may also be precursors to PMIE are not clear. The ability to identify and measure robust, repeatable markers of inflammation integral to MIE may be key to understanding the progression to PMIE. The aim of the study was to (i) refine a protocol for inducing MIE and in doing so test a range of cellular and molecular parameters as valid markers of MIE to facilitate future studies of mares susceptible to PMIE (ii) concurrently identify those parameters with potential as inflammatory indicators during MIE to inform and enhance early treatment regimens in practice. Mating-induced endometritis was induced in pony mares using a stringent protocol; mares were treated intrauterine with frozen/thawed semen ( $n = 5$ ; FTS) or frozen/thawed extender ( $n = 6$ ; FTE<sub>x</sub>). The parameters tested were measured before treatment were compared to samples collected at strategic time points after treatment: uterine cytology using cytological (at 8, 16, 24, 48 and 72 h after treatment) or histological analysis (at 24 and 72 h); uterine bacteriology (at 24 and 72 h); secretion of prostaglandin  $F_{2\alpha}$  ( $PGF_{2\alpha}$ ; at 8, 16, 24, 48 and 72 h); peripheral concentrations of serum amyloid A (SAA; at 24h); endometrial mRNA gene expression, focussing upon *IL8* and *TLR4*, as examples of genes pertinent to inflammation (at 24 h). Uterine neutrophil cell numbers in both treatment groups increased at 8 ( $P < 0.001$ ), 16 ( $P < 0.01$ ) and 24 ( $P < 0.01$ ) h after insemination, indicative of MIE and distinguished between different treatments because neutrophil numbers were greater from FTS mares than FTE<sub>x</sub> mares 8h after challenge. Uterine neutrophil cell numbers, assessed by histology, increased ( $P < 0.001$ ) 24 and 72 h after treatment. Prostaglandin  $F_{2\alpha}$  concentrations increased ( $P < 0.05$ ) 16 h after treatments, while SAA concentrations and bacterial growth scores were not significantly different after treatment. Endometrium from pony mares expressed mRNA for *IL8* and *TLR4* but expression was not altered after insemination. The protocol induced MIE, as confirmed by uterine cytology and maybe used hereafter as a repeatable and robust method for studying immune mechanisms that underlie MIE and so may aid the understanding of progression to persistent inflammation. It can be concluded that of the range of parameters tested, neutrophil cell numbers by cytological analysis and  $PGF_{2\alpha}$  were regarded as the most accurate markers of inflammation during MIE and important for use in practice.

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## Endometrial explant culture to study the response of equine endometrium to insemination

Nash, D.M.<sup>1,2</sup>, Sheldon, I.M.<sup>1</sup>, Herath, S.<sup>1</sup>, Lane, E.A.<sup>3</sup>

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### Reproduction in Domestic Animals 45, 670–676 (2010)

Mating-induced endometritis (MIE) is ubiquitous in the horse after natural mating and artificial insemination with frozen/thawed semen causing the most aggressive response. The majority of mares eliminate MIE 24–48 h after insemination. An endometrial explant culture was tested as a potential *in vitro* exemplar for sperm-induced MIE. Endometrial prostaglandin  $F_{2\alpha}$  (PGF<sub>2 $\alpha$</sub> ) secretion and expression of interleukin-8 (*IL-8*) were used as markers of inflammation. Endometrial explants were cultured from uteri collected from follicular phase mares. Explants were challenged with 1 or 10 × 10<sup>6</sup> sperm/ml frozen/thawed semen, chilled semen, washed sperm or seminal plasma. Medium was collected 24 and 72 h after challenge and assayed for PGF<sub>2 $\alpha$</sub>  by radioimmunoassay. Treatment of endometrial explants with frozen/thawed, chilled semen or washed sperm did not change the secretion of PGF<sub>2 $\alpha$</sub>  compared with untreated controls. However, 24 h after challenge cultured explants expressed *IL-8*. The *in vitro* endometrial explant system did not represent the *in vivo* response to semen when PGF<sub>2 $\alpha$</sub>  was used as a marker of inflammation, yet the use of gene expression as an inflammatory marker warrants further investigation.

*Reprinted from Reproduction in Domestic Animals, 45, Nash, D.M., Sheldon, I.M., Herath, S., Lane, E.A., Endometrial explant culture to study the response of equine endometrium to insemination, 670–676, Copyright 2010, with permission from John Wiley and Sons.*

## Essential elements and heavy metal concentrations in a small area of the Castlecomer Plateau, Co. Kilkenny, Ireland: Their potential to impact on animal performance

Canty, M.J.<sup>1</sup>, McCormack, S.<sup>2</sup>, Lane, E.A.<sup>1</sup>, Collins, D.M.<sup>1</sup>, More, S.J.<sup>1,3</sup>

<sup>1</sup> UCD CVERA, <sup>2</sup> Teagasc Johnstown Castle, <sup>3</sup> UCD School of Veterinary Medicine

Many ruminants are solely or mostly dependant for their nutrients, including essential elements, on the forage available to them, either in its natural state or conserved as hay or silage. A soil and herbage survey was carried out in April and September 2007, in a 3.1 km × 3.0 km grid, incorporating 106 and 46 sampling points, respectively, on the Castlecomer Plateau, Co. Kilkenny, Ireland. The aim was to determine the nutrient and heavy metal status of soil and herbage in the sampling area, and to examine the concentrations observed for their potential to impact on animal performance. Low soil pH and high soil lime requirements were identified within the sampling area. The concentrations of Ca, Cu, Se and Zn were low in soils and herbage. These conditions are similar to those found on other farms in Ireland. Fluoride was detected in 61 of the 97 herbage samples in April 2007, but only four exceeded 40 mg/kg dry matter, the maximum tolerable level for cattle. Mineral imbalances (Ca, Cu, Se and Zn) observed in pastures caused by low soil mineral status, exacerbated by low soil pH, could impair animal performance in the area studied.

## Small area study/milk recording data

Lane, E.A.<sup>1</sup>, Canty, M.J.<sup>1</sup>, Clegg, T.A.<sup>1</sup>, More, S.J.<sup>1,2</sup>

<sup>1</sup> UCD CVERA, <sup>2</sup> UCD School of Veterinary Medicine

The aim was to determine the influence of local industry on milk production in surrounding farms. Data were collected from all dairy farms ( $n = 14$ ) participating in a national milk recording scheme within 8 km of a brick factory. Herd identifier, individual cow identifier, date of birth, most recent calving date, parity, date of test, 24 hour milk, fat, protein

and lactose yield, somatic cell count at each milk recording event ( $n = 13,773$ ). A herd mean 24 hour estimated milk yield was calculated per herd per month over the six year period ( $n = 557$ ). There was a significant effect of herd of origin ( $P < 0.0001$ ), parity ( $P < 0.05$ ) and number of days in milk ( $P < 0.01$ ) on the herd average milk yield. Mean milk yields were lower ( $P < 0.01$ ) on the index farm compared with all farms with the exception of herds 5 and 10. There was no effect ( $P > 0.05$ ) year, or number of cows in herd, or somatic cell counts on herd average milk production. Milk yield was highest ( $P < 0.001$ ) in second quarter of year and lowest ( $P < 0.001$ ) in fourth quarter of year. Univariate analysis demonstrated that herds located within 5 km of the brick manufacturing unit, milk production was lower ( $P < 0.0001$ ) when the brick manufacturing unit was in production ( $18.8 \pm 0.37$  kgs/day) compared with when it was closed ( $21.7 \pm 0.41$  kgs/day). Milk production was not different for herds that were located between 5 to 8 km from the unit. Mixed model analysis will be conducted to determine the overall effect of brick manufacture, accounting for biologically important factors including herd of origin, parity and time of year, on milk production in the surrounding herds.

## Image quality & radiological safety

Skelly, C.<sup>1</sup>, Redmond, B.<sup>2</sup>, More, S.J.<sup>1,3</sup>, McAlister, H.<sup>1</sup>

<sup>1</sup> UCD School of Veterinary Medicine, <sup>2</sup> UCD School of Applied Social Science, <sup>3</sup> UCD CVERA

X-rays have long been used in veterinary practice both as a screening tool for developmental conditions and for diagnostic purposes when dealing with sick animals. However there is limited information regarding the radiographic protocols, image quality and radiation safety standards of veterinary practices in Ireland. This project is designed to assess the changes that have occurred in radiographic protocols and image quality over a ten-year period that has seen great technological advancement and to determine the attitudes and perceptions of the personnel involved in veterinary radiography towards radiation safety.

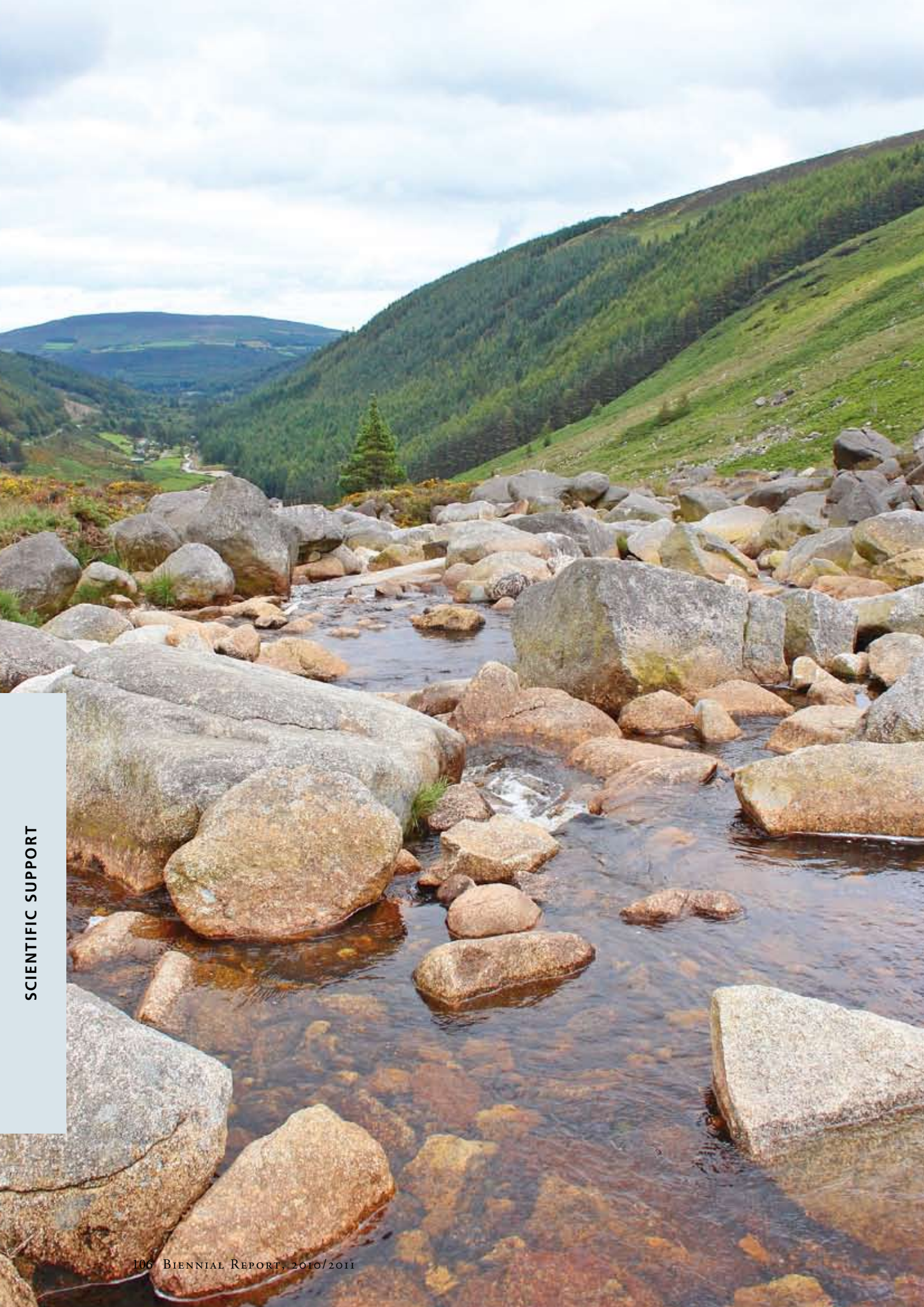
## Can a national fertility programme improve dairy herd performance in Ireland? A review

Lane, E.A.<sup>1</sup>, Crowe, M.A.<sup>2</sup>, More, S.J.<sup>1,2</sup>

<sup>1</sup> UCD CVERA, <sup>2</sup> UCD School of Veterinary Medicine

Dairying is a key element of the Agri-food industry in Ireland. Although the number of dairying units is decreasing, milk productivity and value are increasing. All indicators suggest that farms are set to expand. Reproductive performance is reducing worldwide and infertility is a serious constraint to farm expansion. Intensively managed dairy herds must achieve fertility targets to ensure long-term economic viability. Computer programmes to manage fertility were developed in the sixties and recent advances enable the development of sophisticated integrated computerised programmes for fertility, health and production facilitate herd management. The analysis of herd management records allows for accurate assessment of the current status of the herd, a crucial decision making tool to implement effective change. Monitoring of such changes to ensure their effectiveness is essential to the success of any programme. Furthermore, participation in discussion groups, allows for peer comparisons, a key factor in motivating herd management change. A national programme such as the InCalf programme offers Ireland the opportunity to avail of a highly resourced effective reproductive performance programme that is in operation for similar production systems in the Southern Hemisphere. We must be mindful of the excellent resources already in existence in the country; the ICBF database offers us opportunities to amalgamate the InCalf programme with a key database to further enhance the possible outcomes for our dairy industry. Additionally, successful farmer discussion groups are already operational in Ireland run by our farm advisory service Teagasc. Capacity building must be at the forefront of the programme and the encouragement of farmer, advisor and industry participation is paramount. These are challenges our industry must embrace to enhance the Irish agriculture.









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## Scientific support

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## Epidemiological support

Key CVERA contacts: Simon More, Inma Aznar, Mary Canty, Liz Lane

The UCD Centre for Veterinary Epidemiology and Risk Analysis (CVERA) provides epidemiological support in a wide variety of areas. CVERA staff are active in support of on-farm investigations by undergraduate veterinary students, and of research projects by postgraduate students, at University College Dublin (UCD). Working closely with colleagues in Ireland and internationally, they also collaborate, either in a lead or support role, on a broad portfolio of research projects, contributing epidemiological expertise to the design, conduct, analysis and finalisation of scientific research. CVERA continues to provide epidemiological training to staff of the Department of Agriculture, Food and the Marine (DAFM), through the 6-monthly epidemiological mentoring group. Epidemiological staff in CVERA increasingly work at the science-policy interface, providing scientific advice to support policy decision-making, both in Ireland and internationally. In Ireland, CVERA works closely with government organisations (including DAFM, the Food Safety Authority of Ireland (FSAI), and the Marine Institute) and with Animal Health Ireland (AHI). Internationally, CVERA staff contribute to the work of the European Food Safety Authority (EFSA). In general, epidemiological support is undertaken in collaboration with other CVERA staff, with expertise in statistics, geographic information systems and database maintenance and interrogation.

The following provide an overview of some of the epidemiological support provided by CVERA during the last 2 years:

### *Scientific information in support of national and international policy decision-making*

Most of CVERA's work is conducted at the science-policy interface, with CVERA staff conducting scientific research and providing scientific information in support of policy decision-making.

In Ireland, CVERA works very closely with DAFM, principally in the broad area of regulatory animal health (including bovine tuberculosis, emergency disease preparedness and response, and animal welfare). CVERA staff work in close collaboration with AHI, providing scientific support through Technical Working Groups (TWGs; ongoing chair of the TWGs on John's disease and mastitis, past chair of the TWGs on biosecurity and calf health) and through applied scientific research, focused on key AHI issues. Simon More is a member of FSAI's Biological Safety Subcommittee.

Internationally, Simon More is a member of EFSA's Animal Health and Welfare Panel and has contributed to each of the following AHAW working groups:

- Meat inspection (chair, 2010-)
- Q fever (chair, 2010)
- The use of a gamma interferon test for the diagnosis of bovine tuberculosis (member, 2011-)
- Review of the European Union Summary Report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2010 – specifically for the data related to bovine tuberculosis, Echinococcus, Q fever, brucellosis and non-food borne zoonoses (member, 2011-)
- TB in deer (member, 2009)

Since 2008, he has been a member of the Epidemiology & Wildlife Risks Programme Advisory Sub-Group, which forms part of Defra's Bovine TB Science Advisory Body in the UK.

## Epidemiological teaching

### UCD taught programmes

CVERA staff make a substantial contribution to UCD taught programmes, both to undergraduate and postgraduate students, in the following courses:

#### Veterinary Medicine

- *VET10030 Applications and Integration 1* (development of several problem-based learning exercises, 1<sup>st</sup> year MVB programme)
- *VET30170 Veterinary Herd Health and Population Medicine* (lectures, 4<sup>th</sup> year MVB programme)
- *VET30290 Veterinary Medicine* (practical classes & farm visits, 5<sup>th</sup> year MVB programme)
- *VET40180 Herd Health Investigations Skills* (module coordinator, online learning, Graduate Certificate in Bovine Health Management)
- *VET30070 Clinical reproduction programme* (lectures, 4<sup>th</sup> year MVB programme)
- *VET20020 & VET30390 Equine reproductive physiology* (lectures, 2<sup>nd</sup> year MVB programme)

#### Veterinary Nursing

- *VNUR10240 Veterinary Reception and Management Skills* (lectures, 1<sup>st</sup> year Veterinary Nursing Degree programme)
- *VNUR30310 Introduction to Research* (lecture, 3<sup>rd</sup> year Veterinary Nursing Degree programme)

#### Other taught programmes

- *AESC40020 Epidemiology and Zoonoses* (lecture, Masters students, Agricultural and Environmental Sciences)
- *ANSC30130 Animal health, welfare and behaviour* (lectures, Animal Science students)
- *MDSA10210 Science Medicine and Society* (lecture, Medicine and Medical Science)
- *Advances in Infection Biology* (lecture, Infection Biology Thematic PhD)

### Non-UCD taught programmes

CVERA staff have also contributed to the following:

- *Certificate programme in stud management* (Liz Lane lectures on an approach to foaling mares, Irish National Stud)
- *European Diploma in Animal Reproduction sub species Bovine (DiplECAR (Bovine))*. (Liz Lane was the official supervisor for Marijke Beltman)

## Epidemiological training for DAFM staff

CVERA coordinates an *epidemiological mentoring group*, to support veterinarians with an interest in the practical application of veterinary epidemiology in their work. The group met in Portlaoise, Co. Laois, in May 2010 and in February and November 2011. The meeting includes informal support for fundamental epidemiological issues (for example, 'writing scientific papers', epidemiological study designs' etc). In addition, a group of epidemiological mentors (from CVERA and elsewhere) are providing support to group members on epidemiological projects that they are leading. Some current projects include:

- Seán Ashe, Investigation of oyster mortality in Ireland
- Dorothy Bailey, Investigation of the piroplasmosis outbreak in horses during 2009
- Aidan Cahill, Factors affecting BSE sample quality

- Bosco Cowley, Prevalence of IBR and BVD write-up
- Mary Cullinane, Further analysis of the emergency slaughter data
- Anthony Duignan, Quality control in the national bovine TB programme
- Andrea Dwane, Critical review of the suckler welfare scheme
- Nicky Fennelly, Bovine TB trends in Kilkenny
- Martin Hayes, Correlation between SICCT and gamma-interferon tests
- Damien Kelly, Evaluating the concepts of risk-based surveillance in the national bovine TB programme
- Paddy Kelly, Evaluating an early warning system for on-farm animal welfare incidents
- Peter Mullooney, Management of herpesvirus infection in oysters
- Declan Murray, Analysis of single reactor breakdowns
- John O’Gorman, Analysis of AHCS form 1
- Eoin Ryan, Ongoing epidemiological work with BSE and Q fever

Several of these projects were recently published in the international peer reviewed literature.

## *Animal Health Ireland*

CVERA has worked in close collaboration with a range of other partners towards the establishment of, and subsequently in support of, AHI, a not-for-profit, partnership-based organisation providing national leadership and coordination of non-regulatory animal health issues in Ireland. Partner organisations include livestock farmers, processors, service providers and government. In Ireland, as elsewhere, regulatory animal health issues are the responsibility of government. Until recently, however, there has been no national coordination of non-regulatory animal health issues. AHI was established specifically to fill this gap. For some years, CVERA sought to build a science-base in support of AHI’s formation, providing a critical review of current performance in non-regulatory animal health in Ireland, and building a case for increased private sector involvement in non-regulatory animal health issues. Following AHI establishment in early 2009, CVERA has been heavily committed, with other partners, in providing scientific information in support of national decision-making in this area, through AHI’s technical working groups, focusing on areas including biosecurity, calf health, Johne’s disease and mastitis. CVERA is also leading or contributing to a number of highly applied research programmes in these areas.

## *Irish Veterinary Journal*

In collaboration with Michael Doherty, the Editor-in-Chief, Simon More has worked to establish the *Irish Veterinary Journal* as an open access journal, within the BioMed Central (BMC) stable. BMC currently publish 227 international peer-reviewed journals, all open access, most in partnership with scientific societies. The journal, located at [www.irishvetjournal.org](http://www.irishvetjournal.org), now has the potential to reach its full potential, providing an international showcase for high-quality Irish veterinary medical research. The journal is now indexed widely, including in PubMed, and highly visible internationally. As stated by Michael at its recent “re”-launch:

*“The aspirations and achievements of the Irish veterinary profession and Irish veterinary science are world-class, in terms of patient care, national disease control, veterinary education and research. This exciting new relationship between Irish Veterinary Journal and BioMed Central, providing open access publishing and concomitant increased international standing, is entirely in keeping with those aspirations and achievements.”*

CVERA is leading the IVJ archiving project, which seeks to create a complete electronic archive of the *Irish Veterinary Journal*, from volume 1 in 1946. At this point, all peer-reviewed journal articles from volume 57 (2004) are available online.

## Statistical scientific support

Key CVERA contacts: Tracy Clegg, Mary Canty

CVERA provides statistical support in a variety of areas ranging from study design, statistical analysis, interpretation of results and assistance with publishing results. Support is mainly provided to personnel in DAFM on various research areas. In addition, statistical support is provided to AHI technical working groups as requested and to researchers in UCD Veterinary Medicine and other organisations. Advice and technical expertise is also provided by CVERA for the statistical software packages SAS, STATA, SPSS and, to a lesser extent, WINBUGS.

During 2010-2011, in addition to core projects already described, CVERA provided statistical support and advice to a range of research projects as follows:

### *Department of Agriculture, Food and the Marine (DAFM)*

#### **Routine support**

- Estimation of the potency of tuberculin supplied to Ireland. Around twice each year DAFM carries out trials on cattle that have already tested positive to the Single Intradermal Comparative Tuberculin Test (SICTT) in order to estimate the potency of tuberculin supplied to Ireland. CVERA receives the data from these trials and runs the appropriate models to estimate the potency of the tuberculin batches tested
- Selection of a random sample of herds for a surveillance programme on Bluetongue virus in the Irish cattle population
- Provide statistical support annually to assist in the quantitative comparison of the performance of individual private veterinary practitioners (PVPs) in relation to SICTT testing for bovine tuberculosis

#### **Statistical support**

- Provided statistical assistance with the data collection, descriptive analysis and the use of logistic regression for a study of bovine tuberculosis in animals slaughtered from single reactor breakdown at an Irish export meat plant
- Assisted with the sample selection and analysis of prevalence for a survey of the distribution of paratuberculosis (Johne's disease) in cattle herds in Ireland
- Provided statistical advice for a study of tuberculosis in goats on a farm in Ireland
- Assisted with descriptive analysis of bovine cases consigned under veterinary certification to emergency and casualty slaughter in Ireland during 2006 to 2008
- Advised on the study design to identify non-compliance of sheep identification
- Performed statistical analysis using survival analysis to compare the performance of SICTT testing carried out by TVIs compared with herds tested by pvp's when testing herds for bovine tuberculosis
- Advised on the statistical analysis of the calving interval of cows in 5 beef herds by various risk factors

## UCD School of Veterinary Medicine

### Teaching

- *VNUR30310 Introduction to Research* (lecture, 3<sup>rd</sup> year Veterinary Nursing Degree programme) – lectures and tutorials on statistics

### Statistical support

- Statistical advice provided on the appropriate non-parametric tests to use for the study: Comparison of ultrasound-guided versus “blind” techniques for intra-synovial injections of the shoulder area in horses: scapulohumeral joint, bicipital bursa and infraspinatus bursa
- Assisted with the statistical analysis for a study of a retrospective review of weight loss despite a good appetite in horses
- Statistical advice provided on the appropriate methods to use to compare SCC measurements for the study: A HACCP-based approach to mastitis control in dairy herds. Part 2: Implementation and evaluation
- Assisted with the descriptive data analysis of Delphi results from the study: Evaluation of current equine welfare issues in Ireland: Causes, desirability, feasibility and means of raising standards
- Advice provided on the study design to compare different diagnostic imaging techniques
- Assisted with the statistical analysis to compare biosecurity risks at horse events
- Assisted with the data analysis for a study of persistent-post breeding endometritis in mares comparing swabs taken before and after breeding from the vagina and uterus
- Contributed to a study on the number of herd restrictions due to bovine tuberculosis for a project based on herds in West Wicklow



## Geographic Information Systems (GIS) scientific support

Key CVERA contacts: Guy McGrath, Daniel M. Collins

CVERA provides a broad range of GIS scientific support to research and disease eradication projects run by DAFM. Additionally, GIS support is provided to UCD Veterinary Medicine and other organisations. Listed is a sample of GIS work conducted in CVERA which is not credited formally through peer review.

### *The Wildlife Unit*

#### **a. An independent monitor**

CVERA acts as an independent monitor for the National Parks and Wildlife Services (NPWS) within the Department of Arts, Heritage and the Gaeltacht to ensure operations of the Wildlife Unit (DAFM) are within pre-agreed criteria. This includes verifying individual badger removal licences and calculating a running total of the total area of land under treatment by county. Six-monthly reports are produced for the two government Departments.

#### **b. Administration**

In addition to monitoring and reporting on Wildlife Unit activities, CVERA maintain the GIS component of the Wildlife Unit administration centre in Johnstown Castle, Co. Wexford. This centre provides all District Veterinary Offices with the relevant maps and ortho-photography to complete badger surveys in areas where tuberculosis breakdowns in cattle have been attributed to wildlife. The badger setts found through surveying are digitised and maintained centrally on the GIS. Spatial queries are employed to validate sett location and reduce input errors.

#### **c. Vaccine trial**

A vaccine that protects badgers from bovine tuberculosis is currently being trialled. The field trial area in west Kilkenny was selected based upon location, herd count and cattle density, existing sett density, topology and land use using GIS. Additional locations in other counties have been identified to replace badger culling with vaccination as part of an additional trial. Ongoing GIS support will be provided for the maintenance and analysis of these projects.

#### **d. General Wildlife Unit tasks**

Specific queries and issues considered to be of local importance are dealt with on an *ad hoc* basis. This includes mapping neighbourhood TB testing history to assist in decision making in problem areas. All District Veterinary Offices are supplied with A0 maps annually showing the location of their setts and capture blocks.

### *Technical GIS support*

#### **Administrative**

In the event of an outbreak of a Class A disease, CVERA provide GIS support to the DAFM's National Disease Control Centre. In the case of Foot and Mouth disease (FMD), CVERA liaise with the Irish meteorological office, Met Éireann, to establish the windborne risk of infection to farms in the vicinity of an index case. CVERA are currently involved in the process of unifying FMD zone mapping with our GIS counterparts in the Department of Agriculture and Rural Development, Northern Ireland. CVERA have provided contingency mapping support for diseases associated with bovine, avian, equine, porcine and ovine species.

## Epidemiological

CVERA assist in the spatial components of a diverse array of epidemiological projects. These projects include:

- Selecting random sites nationally for the survey of *Culicoides*
- Herd selection by grid square for annual Bluetongue surveillance
- Cluster analysis of Johne's Disease
- Clustering of *Cryptosporidium*
- Associating local climatic events with poor performance on a study herd
- Aerial dispersion modelling
- Continuation of disease density 'green blob' maps 2007 through to 2010
- Mapping of lead levels in bovines in the Silvermines area

## General administrative GIS support

CVERA provides regular GIS administrative reports to DAFM. Examples of GIS administrative reports to the Department include:

- Estimating the distance between main farms and their corresponding county District Veterinary Office (DVO)
- Calculating the catchment areas for DAFM Veterinary Laboratory Service regional offices
- Calculating DVO farm catchment areas
- Providing mapping assistance for the early stages of the N11 badger tracking project

## Ad hoc GIS support

CVERA provides once-off GIS support to a range of projects conducted by the Department, UCD Veterinary Medicine and other collaborating agencies. Examples of recent GIS support include:

- Creating a map for DVOs showing live transport resting stops in France under EU welfare requirements (DAFM)
- Mapping the submission of equine cases to the UCD Veterinary Hospital (UCD)
- The location of roads studied for a paper on road casualty mammals (UCC)
- The territories and badger sett locations of Little Island, Co. Waterford (UCC)
- Maps illustrating various aspects of the Irish equine industry as part of a publication entitled "Challenges and solutions to support good equine welfare practice in Ireland" (UCD)
- Selection on a Control area for the N11 badger tracking project (DAFM)
- Construction of a national 10 metre Digital Terrain model (DTM) of Ireland
- Creation of a complete seamless Land Parcel Identification System (LPIS) coverage of Ireland from 1999 with spatial attribution applied to the most recent entries
- Trichinella maps and bovine density maps for DAFM Veterinary Laboratory Service
- Piroplasmosis mapping (DAFM)

## *Provision of geographic data*

CVERA provide collaborative agencies with geographic data. Some of these projects include:

- The provision of Land Parcel Identification System (LPIS) to National Parks and Wildlife Services (NPWS) on an ongoing basis
- The provision of Four Area Project data and GIS assistance to The School of Natural Sciences in Trinity College Dublin
- The provision of data to the UCD School of Mathematical Sciences for a project based on herds in the West Wicklow area
- The provision of data for a project entitled “Effect of distance between a previously *Mycobacterium bovis*-infected herd and clear herds on the occurrence of bovine tuberculosis breakdowns in Ireland
- The provision of spatial data to model the badger populations in Ireland
- The provision of data for a project aimed at evaluating a risk-based approach to meat inspection for bovine tuberculosis in Ireland
- The conversion of Irish geographic XY location data to international lat/long standard
- The provision of mapping resources as part of veterinary student herd investigations
- Ann Valley catchment constructed wetland data support
- The provision of data for a national wildlife survey

## Database scientific support

Key CVERA contact: Isabella Higgins

Provision and preparation of data for research projects for CVERA, PhD theses/publications and support for a range of projects from the mentoring group

### *Maintenance and conversion of the following databases to SAS® datasets*

CVERA maintains a copy of the following national databases:

- Animal Health Computer System (AHCS) database
  - TB Herd test summary records
  - TB Animal level records
  - TB Factory surveillance database
- Animal Identification and Movements System (AIMS) database

Each is converted to SAS® datasets, to facilitate data extraction and analysis.

### *Data extraction*

Data extraction from the above-mentioned databases has or is being used for a broad range of projects, including:

- Cost benefit analysis of Irish BVD eradication programme; descriptive data for suckler, dairy and beef fattening herds by herd size
- Calf wastage project to estimate calf mortality in Ireland; data extraction from AIMS and collation with ICBF data from milk-recording herds
- Wind distribution models; provision of assistance with the handling of raw data
- Thematic mapping of bovine TB incidence per square km; provision of APT figures on a DED basis (for kernel density with search radius at 10km) for the years 2010 and 2011
- Genetics of tuberculosis in Irish dairy cows; merging of linkable records from AHCS, AIMS (formerly CMMS, Cattle Movement Monitoring System) and the Irish Cattle Breeding Federation (ICBF) database to create animal and herd level records of animal breeding and disease control
- Provision of demographic data for a retrospective cohort study on the relationship between herd size, herd expansion and milk somatic cell counts
- Provision of demographic data on farms selected for the study on molecular epidemiology of *Cryptosporidium parvum* subtypes using multi-locus subtyping approach and geographic information system approach
- Identify key performance indicators (KPIs) that can be monitored to enhance the Early Warning System (EWS) for Animal Health and Welfare; data extraction from AIMS of the following data: Late registrations, Cattle exits by on-farm burial, Cattle moves to the knackery, Cattle moves to herd E800 and Total cattle exits (move to the factory, abattoir, mart, private sale, knackery, on-farm burial, locate or E800)
- Investigating the long-term survival and movement of BVD antigen positive animals to the knackery/laboratory, factory or other farms; provision of movement data from AIMS for these animals, as confirmed by DAFM Veterinary Laboratory Service during 2010
- Provision of episode based data and movement records as a follow up to previous work on the genetics of tuberculosis in Irish dairy cows. The main objective of this further study is to fine map the regions of the genome associated with TB and to investigate using beef animals and to create more accurate dependent variables (i.e. genetic merit for TB on sires)

## *General database support*

CVERA provides general database support through each of the following:

- Support the structure of epidemiological data storage in SAS®
- Ensure timely and accurate management of epidemiological data
- Lead on-going data maintenance efforts
- Ensure epidemiological data is entered accurately
- Programming in SAS® requests for analyses of epidemiological data
- Assist with SAS® upgrades
- Recommend and assist in the implementation of process improvements for data exchange
- Create, edit and update epidemiological datasets as requested
- Interact with and respond to epidemiological data questions
- Participate in projects and department meetings to maintain awareness of changing needs as they relate to epidemiological data usage
- Identify new epidemiological data requirements and changes to existing epidemiological data

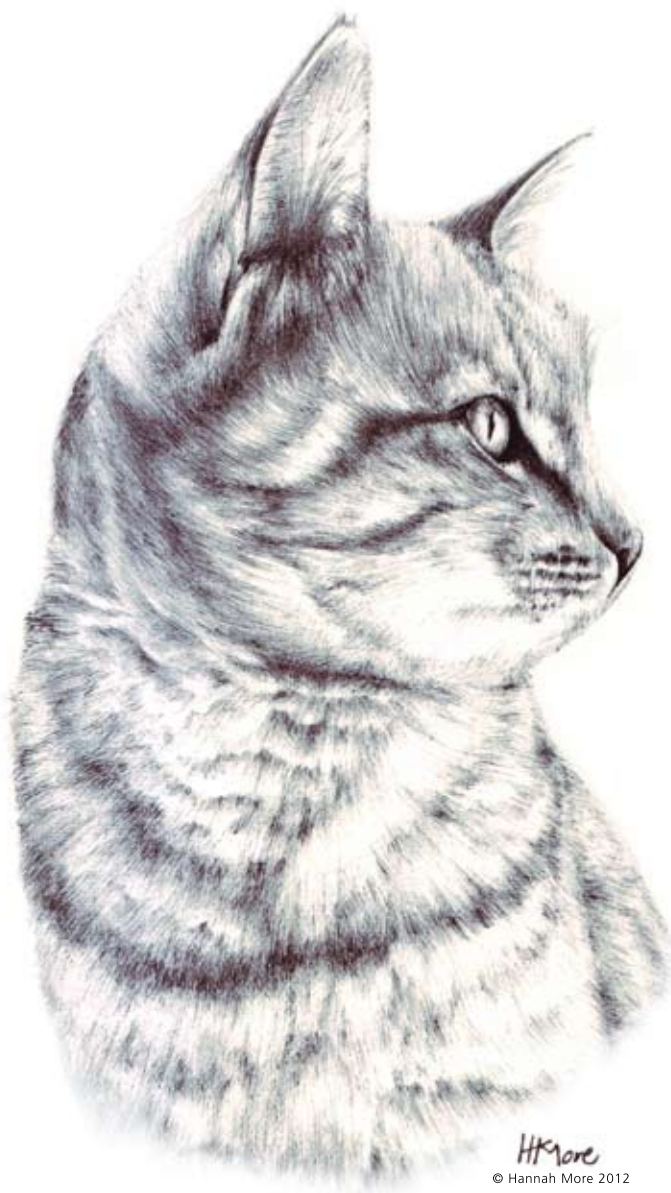
## *Creation of episode-based data*

Detailed work was conducted to create a database of TB breakdown episodes for the period 1989-2010. This work will form the basis of extensive future work in the study of TB, including each of the following research projects:

- Compilation and validation of episode-based data for the Restocking Study and identification of the study population
- Provision of extensive tabulated data over the period 1995-2010 of bovine TB trends in Ireland as part of work in collaboration with England, Northern Ireland, Scotland and Wales using episode-based data, AHCS TB herd test summary records and TB animal test records
- The development and application of new measures of performance, relating to the management of the Irish TB eradication programme. This project will assist with ongoing programme review to enable ongoing measurement and to review programme progress







Publications

*During 2010-11* ..... 120

*Prior to 2010* ..... 128

## During 2010-2011

### Peer reviewed papers

Aznar, I., McGrath, G., Murphy, D., Corner, L.A.L., Gormley, E., Frankena, K., More, S.J., Martin, W., O'Keeffe, J., De Jong, M.C.M., 2011. Trial design to estimate the effect of vaccination on tuberculosis incidence in badgers. *Veterinary Microbiology* 151, 104-111.

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Beekhuis-Gibbon, L., Devitt, C., Whyte, P., O'Grady, L., More, S.J., Redmond, B., Quin, S., Doherty, M.L., 2011. A HACCP-based approach to mastitis control in dairy herds. Part 2: Implementation and evaluation. *Irish Veterinary Journal* 64, 7.

Beekhuis-Gibbon, L., Whyte, P., O'Grady, L., More, S.J., Doherty, M.L., 2011. A HACCP-based approach to mastitis control in dairy herds. Part 1: Development. *Irish Veterinary Journal* 64, 2.

Bermingham, M.L., Brotherstone, S., Berry, D.P., More, S.J., Good, M., Cromie, A.R., White, I.M.S., Higgins, I.M., Coffey, M., Downs, S.H., Glass, E.J., Bishop, S.C., Mitchell, A.P., Clifton-Hadley, R.S., Woolliams, J.A., 2011. Evidence for genetic variance in resistance to tuberculosis in Great Britain and Irish Holstein-Friesian populations. *BMC Proceedings* 5(Suppl 4), S15.

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Berry, D.P., Good, M., Mullowney, P., Cromie, A.R., More, S.J., 2010. Genetic variation in serological response to *Mycobacterium avium* subspecies *paratuberculosis* and its association with performance in Irish Holstein-Friesian dairy cows. *Livestock Science* 131, 102-107.

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# CVERA Board of management

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\*\* UCC School of Biological, Earth & Environmental Sciences

\*\*\* School of Natural Sciences, Trinity College Dublin

## Scientific opinions

(J.D. Collins [UCD CVERA] and J.M. Griffin [DAFM] with other members of the Scientific Panel on Biological Hazards (BIOHAZ), and S.J. More [UCD CVERA] with other members of the Panel on Animal Health and Welfare (AHAW) of the European Food Safety Authority [EFSA])

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# CVERA Board of management

\* DAFM Veterinary Laboratory Service

\*\* UCC School of Biological, Earth & Environmental Sciences

\*\*\* School of Natural Sciences, Trinity College Dublin

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(J.D. Collins [UCD CVERA] and J.M. Griffin [DAFM] with other members of the Scientific Panel on Biological Hazards (BIOHAZ) and S.J. More [UCD CVERA] with other members of the Panel on Animal Health and Welfare (AHAW) of the European Food Safety Authority [EFSA])

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